COMMONWEALTH MYCOLOGICAL INSTITUTE
12 APR 1960

New Zealand Journal of Agricultural Research

VOLUME 1
1958

Digitized by the Internet Archive
in 2023

https://archive.org/details/newzealandjourna0001na

New Zealand Journal
of
Agricultural Research

VOLUME I No. I
FEBRUARY 1958

18 MAR 1958

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind, however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. 4¼ × 7 in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is 4¼ × 7 in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation, etc., of references, see examples in this issue.

NEW ZEALAND JOURNAL
of
AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. A. Black.

VOL. 1. FEBRUARY 1958 Number 1

EFFECTS OF DIFFERENCES IN LIGHT INTENSITY AND TEMPERATURE ON THE ANATOMY AND DEVELOPMENT OF LEAVES OF *Lolium perenne* AND *Paspalum dilatatum*

K. J. MITCHELL and KATHLEEN SOPER*, Grasslands Division,
Department of Scientific and Industrial Research,
Palmerston North.

(*Received for publication, 11 September 1957*)

Summary

Plants of *Lolium perenne* and *Paspalum dilatatum,* which were germinated under uniform conditions, were grown subsequently under two contrasting conditions of light and temperature: (a) full daylight at a mean temperature of approximately 14°C, (b) shading to 20% of daylight at a mean temperature of approximately 24°C. At the time the fifth leaf had emerged some of each set of plants were transferred to the contrasting conditions.

Under shading at higher temperature, early formed leaf blades were approximately twice the length and half the width of those matured in full daylight at the lower temperature and differences of similar magnitude were present in subsequently formed leaves. However, in plants for which the treatments were later reversed, leaves which had begun to elongate beyond the stem apex at the time of reversal finally had dimensions similar to those of leaves formed and grown to maturity under the contrasting conditions. From these various results it was deduced that size of stem apex and of primordium had little influence on the dimensions of the leaf at maturity.

The two contrasting treatments did not induce any large differences in the dimensions of the stem apices. Differences between circumferences of the stem apices under the two treatments were apparently negligible in *L. perenne*, and with *P. dilatatum* did not reach significance ($p = 0.09$). For *L. perenne* leaf width was determined largely by the circumference of the sub-apical meristematic zone of the stem. An increase in circumference of this meristematic zone arose primarily from an increase in cell number.

---

*Now Mrs. C. S. W. Reid.

N.Z. J. agric. Res. 1; 1-16.

Under shading at high temperature, as compared with full light at lower temperature, the leaf blades had a higher proportion of epidermal and other supporting and conducting tissues and a lower proportion of mesophyll. Cells of the lower epidermis were longer but of smaller volume. Vessel members, cells of the outer bundle sheath, and cells of the upper epidermis also were longer. The volume of the mesophyll cells was less and the mesophyll tissue less compact.

INTRODUCTION

Large morphological differences in the vegetative shoots of *Lolium perenne* and *Paspalum dilatatum* can be rapidly induced by changes in light intensity, temperature, or defoliation (Mitchell 1953, 1955). These are partly due to differences in the number of tillers developed and partly to changes in the dimensions of the leaves, which in the vegetative phase of development constitute almost the whole of the shoot tissue.

The studies reported in this paper were undertaken to determine, firstly, what differences in leaf anatomy accompany the changes in dimensions of the mature leaf, and secondly where the differences arise during the development of the leaf.

Inter-relationships between leaf form and the development of the precursor meristematic tissue have been considered by a number of workers. Abbé *et al.* (1941) found that the increase in width of successive leaves on the main stem of *Zea mays* is correlated with an increase in size of the stem apex, that is, of the dome of tissue above the last-initiated leaf primordium. Wardlaw (1952) from experimental studies with pteridophytes concluded that the diameter of the shoot and the size and shape of the leaves are largely determined by the nutritional status, and hence the size, of the subapical region, the primary morphogenetic developments which take place in the apical meristem remaining virtually unchanged.

As a rise in temperature generally intensifies the effects of a reduction in light intensity on form of shoot development (Mitchell 1955), the comparisons made in the experiments reported here were between plants grown at high light intensity with low temperature and plants grown under shading at a high temperature. For brevity, these treatments are given in the text as "full light" and "shaded" respectively.

The general anatomy of the plants, which is basically similar to that of the graminaceous types examined by Sharman (1942, 1945), is described elsewhere (Soper and Mitchell 1956, Soper 1956).

METHODS

Seeds of *Lolium perenne* and *Paspalum dilatatum* were germinated under uniform conditions of light and temperature in fertile soil in glazed earthenware pots. Just prior to the emergence of the first leaf through the coleoptile, the plants were divided into two groups of equal size which were then grown under contrasting conditions of (a) full autumn daylight at a mean temperature of approximately 14°C., and (b) shaded to 20% of full daylight at a mean temperature of approximately 24°C.

When the fifth leaf on the main stem had appeared, the plants in each set of conditions were divided into three groups, each group containing six plants. One group was harvested for examination, the second remained under the original conditions, while the third was transferred to the contrasting conditions. All plants in the second and third groups were harvested when the eighth leaf on the main stem had appeared.

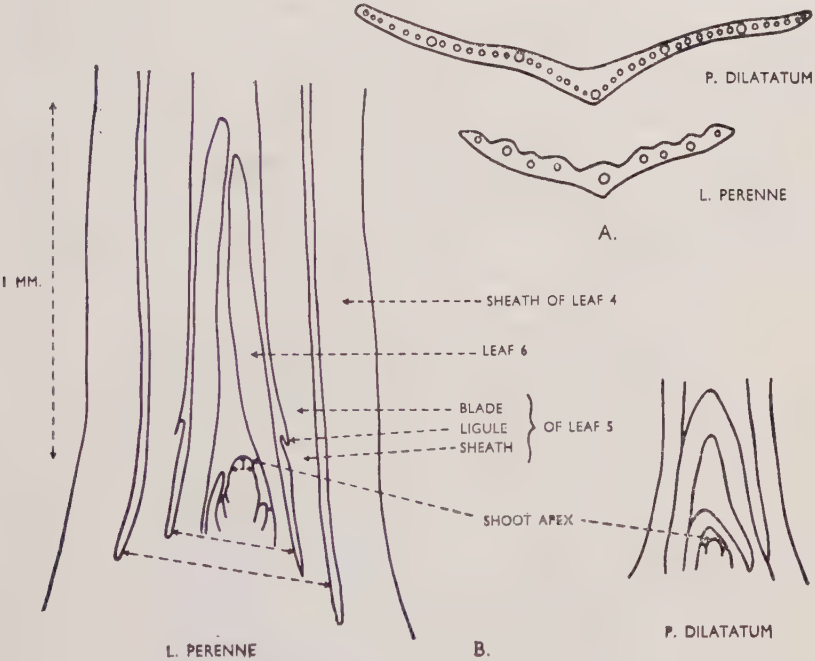


Fig. 1.—A. Outline drawings of cross-sections of the mature leaf blades of *P. dilatatum* and *L. perenne*.

B. Outline drawings of longitudinal sections through the stem apices and developing leaves of *L. perenne* and *P. dilatatum*. The positions at which measurements were made of the apex and sub-apical region of the stem of *L. perenne* are indicated.

For *L. perenne*, measurements were made of blades of mature leaves on the main stem, of intercalary meristems of developing leaves, of the stem apex, and at two positions in the meristematic region subtending the apex (Fig. 1). For *P. dilatatum*, measurements were made of blades of mature leaves and of the stem apex. In vegetative plants of both species there is usually no elongation of internodes and there is a region of meristematic tissue extending down from the apex across the top of the stem and up into the intercalary meristems at the base of the developing leaves. Since the stem apices of vegetative plants of both species are approximately hemispherical, the dimensions of each were measured from a point where the distance from the top of the apex was equal to the horizontal radius of the apex. It was considered that this gave a standard position for measurement which was not influenced by whether a leaf primordium had just developed or was just about to develop. Examination of cross-sections of apices indicated that they were, at the top, sufficiently close to spherical for it not to be essential to have uniform orientation when measuring diameters at the level specified.

Longitudinal and transverse sections were cut of all tissues examined. Material for sectioning was fixed in a mixture of 5 parts formalin, 5 parts acetic acid, and 90 parts 70% ethyl alcohol. After fixation the material was dehydrated and embedded in paraffin wax by the tertiary butyl alcohol method. Although modifications of the formalin-acetic-alcohol and other fixatives were tried, there was always some buckling of the lateral walls of epidermal cells, especially in *L. perenne*.

Sections were cut 8–10 μ thick. Leaf sections were stained in safranin, and apices and meristematic tissues in tannic acid and iron alum with safranin and orange G (Sharman 1943). Cell and tissue dimensions were measured direct from sections or from drawings made with the aid of a micro-projector, except for length and width of cells of the lower epidermis of *L. perenne* which were measured from strips of epidermis. Dimensions of the vessel members and mesophyll cells of *P. dilatatum* were not recorded owing to the difficulty of obtaining accurate measurements. For the same reason, only those cells of the mesophyll adjacent to the epidermis were measured in *L. perenne*.

RESULTS

The effects of the two contrasting treatments on the form of growth of plants of the two species are illustrated in Fig. 2. While "full light" plants had short, relatively broad leaves and numerous tillers, the "shaded" plants had long narrow leaves and no tillers developed.

The Leaf Blade

The dimensions of the leaf blade are set out in Table 1 for both species. At the time the different treatments were begun, the second leaf on the main stem of *L. perenne* had just commenced to elongate beyond the stem apex. Yet the mature blade of the second leaf on the main stem of "shaded" plants of *L. perenne* was twice the length but only half the width and half the thickness of that of the corresponding leaf on "full light" plants. In *P. dilatatum*, relative differences in length and width of the blade of the third leaf were even greater, but the differences in thickness were less.

Similar differences were present in succeeding leaves on the main stem. In plants for which the treatments were reversed when the fifth leaf on the main stem had appeared, the dimensions of the leaves which emerged after the change were more nearly characteristic of the conditions under which they grew to maturity than of those under which they were initiated. Although the sixth leaf had elongated beyond the apex before the change of treatment, it had dimensions similar to those of a leaf grown continuously under the later conditions.

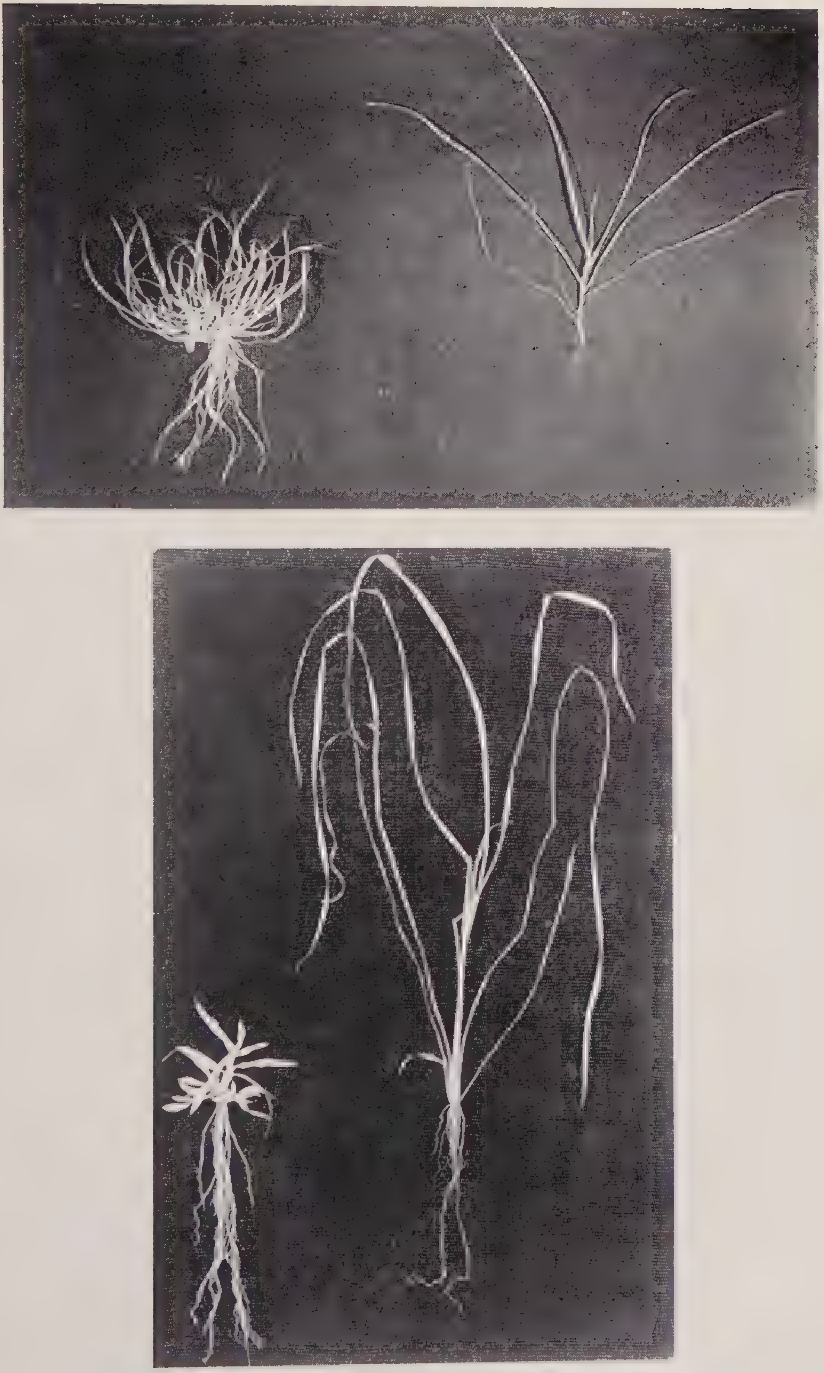


Fig. 2.—Plants of *L. perenne* (above) and *P. dilatatum* (below) grown in "full light" (left) and "shaded" (right) treatments.

TABLE 1. EFFECTS OF CONTRASTING CONDITIONS OF LIGHT AND TEMPERATURE ON DIMENSIONS OF LEAF BLADES
(Means of 6 measurements)

		L. perenne				*P. dilatatum*	
			Sixth Leaf			Sixth Leaf	
Dimension	Final Treatment	Second Leaf	Continuously under Same Treatment	Treatment Reversed When 6th Leaf Commencing Elongation	Third Leaf	Continuously under Same Treatment	Treatment Reversed When 6th Leaf Commencing Elongation
Length (cm)	Full light, 14°c	6.4 **	10.7 **	12.9 **	2.2 **	3.8 **	8.8 **
	Shaded, 24°c	14.3	31.3	24.1	18.8	36.5	19.8
Width at base (cm)	Full light, 14°c	0.31 **	0.45 **	0.45 **	0.38 **	0.72 **	0.67 **
	Shaded, 24°c	0.15	0.32	0.26	0.12	0.36	0.28
Area of blade (sq. cm)	Full light, 14°c	1.39	3.69 **	4.33 **	0.67 **	2.20 **	4.84
	Shaded, 24°c	1.63	9.02	5.94	2.19	16.68	5.90
Thickness at base (cm)†	Full light, 14°c	0.023 **	0.025 *	0.028 **	0.015 **	0.017	0.016 **
	Shaded, 24°c	0.011	0.018	0.016	0.011	0.015	0.011
Cross sectional area at base (sq. cm)	Full light, 14°c	0.0059 **	0.0089 **	0.0098 **	0.0056 **	0.0118 **	0.0103 **
	Shaded, 24°c	0.0015	0.0050	0.0035	0.0014	0.0061	0.0035

* Differences significant at 5% level.
** Differences significant at 1% level.
† Measured through the largest lateral vein.

TABLE 2. PROPORTIONS OF TISSUES IN CROSS-SECTION OF BASE OF LEAF BLADE (percentage of cross-sectional area occupied) $n = 6$

Tissue	Final Treatment	*L. perenne*			*P. dilatatum*			Average S.E. of Means
		Second Leaf	Sixth Leaf		Third Leaf	Sixth Leaf		
			Continuously Under Same Treatment	Treatment Reversed when Sixth Leaf Commencing Elongation		Continuously Under Same Treatment	Treatment Reversed When Sixth Leaf Commencing Elongation	
Epidermis, sclerenchyma, and parenchyma	Full light, 14°c	33	31	29	43	40	44	±1.1
	Shaded, 24°c	42	34	34	57	56	53	±1.7
Mesophyll (including bundle-sheath cells containing chloroplasts)	Full light, 14°c	64	66	67	50	54	50	±1.0
	Shaded, 24°c	53	61	59	34	37	39	±1.3
Vascular bundles	Full light, 14°c	3	4	4	7	6	6	±0.3
	Shaded, 24°c	5	5	6	9	7	8	±0.5

The proportions of the cross-sectional area of the base of the leaf blade occupied by different types of tissues are recorded in Table 2. The "shaded" plants had a higher proportion of epidermal, parenchymatous, and sclerenchymatous tissue, and of vascular tissue, though these differences were not all statistically significant. The proportion of mesophyll was correspondingly lower.

As with leaf dimensions in plants for which the treatments were reversed, the proportions of tissues in the leaves developed after the change were characteristic of the final treatment.

There were also differences in the dimensions of cells in the leaf blade (Table 3). Data are presented only for the second or third leaves on the main stem. Data for the sixth leaf showed similar differences.

For *L. perenne* "shaded" conditions increased the length of the cells of the lower epidermis near the base of the leaf blade by 50% and decreased their depth by 40%. The cells were also slightly narrower ($p = 0.06$). Their volume was thus nearly 20% less than the volume of cells from "full light" plants. In *P. dilatatum* the relative differences in epidermal cell length and width were greater than in *L. perenne* but the differences in cell depth and volume were less marked.

The relative differences in cell size in the lower epidermis are not representative of differences in all tissues of the blade. Although vessel members and bundle sheath cells of *L. perenne* were longer in the "shaded" leaves, the relative differences were less than in cells of the lower epidermis. There was no difference in length (measured along the leaf) of the mesophyll cells in *L. perenne*, but their approximate volume was less in "shaded" leaves. In *P. dilatatum*, the relative difference in bundle-sheath cell length, as in epidermal cell length, was greater than in *L. perenne*.

In both species, there were fewer cells across the blade and more along it in the "shaded" leaf. The relative differences were greater in *P. dilatatum*.

To supplement these results and provide information on the influence of defoliation and of shading without change of temperature, measurements were made of the dimensions of the cells of the lower epidermis of plants from another experiment carried out at the same time (Mitchell and Coles 1955). The plants in this experiment were of a different strain of ryegrass (*L. perenne* × *L. multiflorum*) but its response to the treatments under discussion is similar to that of *L. perenne* (Mitchell 1954). For the treatment conditions used, shading in comparison with full light gave approximately 50% increase in length of the cells of the lower epidermis (Table 4). There was little change in width of these cells. On the other hand defoliation reduced the length of the lower epidermal cells by approximately 30%. It also substantially reduced their width.

The Meristems

Measurements made of apices of plants harvested at the time treatments were reversed are presented in Table 5. The "shaded" conditions

TABLE 3. DIMENSIONS OF CELLS OF LEAF BLADE AND NUMBER OF CELLS IN LOWER EPIDERMIS

Means and standard errors given for cell dimensions (μ), n in brackets.

	L. perenne (Leaf 2)		*P. dilatatum* (Leaf 3)	
	Full light, 14°c	Shaded, 24°c	Full light, 14°c	Shaded, 24°c
Lower epidermal cells				
Length (at base of blade) (60)	455 ± 17	705 ± 24	105 ± 3.6	197 ± 6.6
Width (at base of blade) (30)	23 ± 0.7	21 ± 0.7	23 ± 0.4	16 ± 0.6
Depth (at base of blade) (30)	30 ± 1.1	18 ± 0.6	23 ± 0.4	16 ± 0.6
Volume ($\mu^3 \times 1000$)	314	266	56	50
Vessel members				
Length (30)	468 ± 22	586 ± 19		
Outer bundle sheath cells				
Length (120)	60 ± 1.4	84 ± 1.7	23 ± 0.6	61 ± 1.8
Mesophyll cells				
"Length" (measured along leaf) (240)	23 ± 0.3	23 ± 0.3		
"Width" (measured across leaf) (60)	24 ± 0.5	21 ± 0.6		
"Depth" (60)	34 ± 0.6	19 ± 0.4		
Estimated number of lower epidermal cells				
Across base of blade	135	73	173	71
Along blade	141	211	210	954

TABLE 4. EFFECTS OF DEFOLIATION AND OF SHADING WITHOUT CHANGE OF TEMPERATURE ON LENGTHS AND WIDTHS OF CELLS OF THE LOWER EPIDERMIS OF SHORT-ROTATION RYEGRASS

Means and standard errors

	Glasshouse 24°c Undefoliated	Glasshouse 24°c Defoliated	Glasshouse 24°c Base-shaded
Length (µ) ($n > 40$)	672 ± 35	456 ± 12	982 ± 47
Width (µ) ($n > 60$)	26 ± 0.4	17 ± 0.3	26 ± 0.5

induced a small reduction in circumference of the apex of *P. dilatatum* ($p = 0.09$), but had a negligible effect with *L. perenne*. For both species, differences in the size and numbers of cells in the apex were small or absent.

TABLE 5. DIMENSIONS OF STEM APEX—AS MEASURED FROM LONGITUDINAL SECTIONS

Means and standard errors given, $n = 6$

	Treatment	*L. perenne*	*P. dilatatum*
Circumference (mm)	Full light, 14°c	0.226 ± .018	0.151 ± .010
	Shaded, 24°c	0.214 ± .013	0.126 ± .008
Number of cells across diameter	Full light, 14°c	9.0 ± 0.4	6.2 ± 0.5
	Shaded, 24°c	8.3 ± 0.5	5.5 ± 0.2
Number of cells from tip to level of measurement	Full light, 14°c	5.0 ± 0.2	3.2 ± 0.3
	Shaded, 24°c	5.0 ± 0.2	3.0 ± 0.2
Cell width (µ)	Full light, 14°c	8.0 ± 0.5	7.6 ± 0.2
	Shaded, 24°c	8.2 ± 0.4	7.2 ± 0.3
Cell height (µ)	Full light, 14°c	7.2 ± 0.4	7.5 ± 0.5
	Shaded, 24°c	6.8 ± 0.4	6.6 ± 0.7

For plants of *L. perenne* from both treatments, measurements were made of circumferences of the sub-apical region and intercalary meristems at the time when leaf 5 had just emerged. The ligule of leaf 5 was then within 0.2 mm of the stem apex (Fig. 1). Table 6 records the ratios of circumferences at various positions.

Relative differences between plants from "full light" and "shaded" conditions were small at the stem apex, but much greater in the lower sub-apical region. That the relative differences between widths of the

TABLE 6. RATIOS OF CIRCUMFERENCES AT SELECTED POSITIONS IN THE MERISTEMATIC REGIONS AND OF THE WIDTHS OF BASES OF MATURE LEAF BLADES OF *L. perenne* UNDER THE TWO TREATMENTS

Measurement Position	Ratio of Circumferences—Full light, 14°c Shaded, 24°c	Significance Level for Differences
Stem apex	1.06 : 1	p = 0.60
Base of immature leaf 6	1.39 : 1	p < 0.001
Base of immature leaf 5	1.50 : 1	,,
At level of tip of ligule on immature leaf 5	1.69 : 1	,,
Base of blade of mature leaf 2	2.07 : 1	,,
Base of blade of mature leaf 6	1.41 : 1	,,

mature leaf blades were largely determined at this level is shown by comparing the ratios of circumferences with the ratios of widths of the bases of the leaf blades at later stages of growth. Also it can be seen from Table 7 that where treatments were reversed, there was a corresponding reversal in the dimensions of the sub-apical region.

TABLE 7. CIRCUMFERENCES AT BASES OF IMMATURE LEAVES 8 AND 9 FROM PLANTS OF *L. perenne* ON WHICH TREATMENTS WERE REVERSED WHEN LEAF 5 HAD EMERGED

Means and standard errors

Measurement Position	Circumference (mm) Plants transferred from shade at 24°c to full light at 14°c	Circumference (mm) Plants transferred from full light at 14°c to shade at 24°c	Ratio of circumferences -Full light at 14°c to shade at 24°c
Base of leaf 8 (emerged but immature) ($n = 6$)	2.25 ± 0.25	1.27 ± 0.12	1.77 : 1
Base of leaf 9 (not emerged but elongated above stem apex) ($n = 5$)	1.54 ± 0.32	0.91 ± 0.09	1.69 : 1

It can be deduced from a consideration of the ratios of the circumferences at the bases of the developing leaves, in relation to the differences in numbers of lower epidermal cells across the base of the mature leaf blades, that the differences in circumference of the subapical meristematic region were due more to an increase in cell numbers than in cell width. Similar conclusions can be drawn from measurements made on some of the plants (Table 8).

TABLE 8. NUMBERS AND WIDTHS OF CELLS OF THE LOWER EPIDERMIS ACROSS THE LEAF OF *L. perenne*

Standard errors given for cell numbers; $n = 3$

	Base of Leaf 6 (very young blade)	Base of Leaf 5 (immature sheath)	Above Ligule of Leaf 5 (maturing blade)	Adult Leaf 5 (mature upper part of blade)
Cell no. across leaf				
Full light, 14°c	155 ± 11	310 ± 22	195 ± 9	170 ± 10
Shaded, 24°c	103 ± 6	190 ± 7	130 ± 10	117 ± 11
Cell width (μ)				
Full light, 14°c	6.1	7.2	10.8	23
Shaded, 24°c	5.9	6.6	10.3	21

Leaf length is determined by the number of cell divisions perpendicular to the axis of the leaf and by the extent of subsequent cell elongation. In the lower epidermis of the leaf of the "shaded" plant there were more cells along the leaf, and they were of greater length than in the "full light" plant (Tables 1 and 3). Under these conditions there must therefore have been more cell division and greater cell elongation during the growth of the leaf.

To follow the development of the cells in various tissues of the leaf blade, measurements were made on the immature leaf 5 of *L. perenne*. Although the upper portion of this leaf had emerged from the sheath of the leaf below and was mature, the mature portion graded into a meristematic zone at the base of the blade, and the ligule itself was still less than 1.0 mm above the point of insertion of the leaf on to the stem. Hence the measurements at successive positions up the leaf blade represent successive stages in the development of the leaf tissue to maturity.

Cell enlargement commenced close to the base of the intercalary meristem. At the level of the tip of the ligule, cells were nearly twice the size of those at the base of the leaf. However, as with tobacco (Avery 1933) and apple (MacDaniels and Cowart 1944), the phases of cell division and cell enlargement last for varying periods in different (although often immediately adjacent) tissues of the leaf. Only data for the cells of the lower epidermis and the adjacent mesophyll layer are presented in Table 9. Trends in dimensions of the cells of the

TABLE 9. CELL DIMENSIONS IN IMMATURE BLADE OF LEAF 5 AND MATURE BLADE OF LEAF 2 OF *L. perenne*

Distance Above Tip of Ligule of Leaf 5	Lower Epidermal Cells				Adjacent Mesophyll Cells			
	Length (μ)		Depth (μ)		Length (μ)		Depth (μ)	
	Full light, 14°c	Shaded, 24°c	Full light, 14°c	Shaded, 24°c	Full light, 14°c	Shaded, 24°c	Full light, 14°c	Shaded, 24°c
0.1 – 0.2 mm	20	15	12	11	12	12	13	13
1.0 mm	49	36	17	15	10	10	17	14
2.0 mm	85	66	21	16	8	9	17	16
3.0 mm	100	74	22	17	8	7	19	18
Blade of mature leaf 2	455	705	30	18	23	23	34	19

vascular bundles and upper epidermis were intermediate between these two extremes.

Above the ligule there was a continuous increase in length of cells in the lower epidermis. This increase was more rapid in "full light" plants. In the layer of mesophyll cells immediately adjacent there was a phase of very rapid cell division in the zone 1-3 mm above the tip of the ligule. Consequently the average length of the mesophyll cells was actually decreasing while the length of the adjacent epidermal cells was increasing, and there was a large increase in the number of mesophyll cells adjacent to each lower epidermal cell. Above this zone of rapid cell division the average length of the mesophyll cells increased.

Increase in depth of the cells occurred early, and at a similar rate in both the lower epidermis and the adjacent layer of mesophyll cells. It appeared to be a little slower in the "shaded" plants than in the "full light" plants.

Intercellular spaces appeared in the central mesophyll of the leaf tissue at a distance 4–5 mm above the stem apex in "full light" plants and 5–6 mm in "shaded" plants. It is considered that cell division in the mesophyll has largely ceased when intercellular spaces appear (Avery 1933). Although the average lengths of the cells of the lower epidermis of leaves from the two treatments were similar at the positions where the intercellular spaces appeared in the mesophyll, the lower epidermal cells ultimately elongated 50% more in the "shaded" plants. Consequently there would be less mesophyll cells per unit distance along the blade in "shaded" plants and hence a higher proportion of intercellular space. This implies that in terms of plant material, as distinct from intercellular space, the decrease in the proportion of photosynthetic tissue in the blade of "shaded" plants was considerably greater than indicated by the measurements of cross-sectional area presented in Table 2.

Intercalary Meristem in P. dilatatum

This was not examined in detail. In this species the edges of the developing leaf overlap so that it more than completely encircles the stem, and the circumference of the stem cannot be taken as a measure of the width of the base of the leaf. Also the two treatments induced a considerable difference in the width of the mature cells of the lower epidermis. Therefore, the width of the mature leaf blade is not as closely related to the circumference of the stem at the point of insertion as it is in *L. perenne*.

DISCUSSION

The plants of *L. perenne* had been germinated under uniform conditions and divided into two groups, one in full daylight at 14°c, and the other shaded to 20% of daylight at 24°c, when the second leaf on the primary tiller would have already commenced to extend beyond the apex. Up to that stage of development there would have been no difference in size of stem apex or of leaf primordium between the two groups. Yet there were large differences in the dimensions of leaf 2 at maturity. The deduction that dimensions of the stem apex and leaf primordium have little influence on the dimensions of the mature leaf was confirmed by the transfer of plants at a later stage of development from shade to full daylight and vice versa. Leaf 6 would have commenced elongation when the transfer occurred, yet at maturity it had dimensions characteristic of the environmental conditions under which it developed, not of those under which the primordium was initiated. The same conclusions can be drawn for *P. dilatatum*.

Measurements at various positions in the sub-apical meristematic region of the stem and in the intercalary meristem of the developing leaves of *L. perenne* provided supporting evidence. They showed that the treatments induced large differences in the circumference of the sub-apical meristematic tissue but relatively little difference in the circumference of the apex itself. With *P. dilatatum* the differences in apex circumference induced by the treatments were apparently larger than with *L. perenne* but they were still small relative to the differences between the final leaf widths. As it can be reasonably assumed that the contrasting environmental conditions used in this experiment induced large differences in the carbohydrate nutritional status of the plants, the results are in accord with Wardlaw's view (1952) that the dimensions of the leaf are strongly influenced by the nutritional status and hence the size of the sub-apical meristematic region.

With vegetative plants of these species, low carbohydrate status induced by either shading or defoliation is promptly associated with a cessation or large reduction in activity of meristematic tissues capable of using a large proportion of the total carbohydrate available to the shoot. Inhibition of the development of axillary buds, a reduction in the circumference of the stem sub-apical and leaf intercalary meristems, and a decrease in the rate of growth of individual leaves (Mitchell 1955) can all occur. These differences in activity of the meristematic region around the top of the stem would tend to minimise the effect of large changes in the carbohydrate nutritional status of the shoot on the quantity of carbohydrate available to stem apices; changes in the size of apices should therefore remain relatively small.

Although the carbohydrate nutritional status of the plants was probably of dominant importance in the experiments described in this paper, recent work by Wardlaw (1957) emphasises that certain hormonal substances can also produce substantial changes in the dimensions of the sub-apical meristematic region.

Similarities in the changes in leaf form induced by shading and by gibberellins (Brian *et al.* 1954) suggest that, particularly in the later stages of leaf development, hormonal substances with similar effect to the gibberellins may play an important role in governing the course of leaf development. Such a view is supported by the contrasting effects with these two species of removing two thirds of the photosynthetic tissue by partial defoliation and of cutting off two thirds of the light available by shading. The defoliation leads to a large reduction in size of the leaf blade, and in length of cells of the lower epidermis, while shading generally increases both. Yet similarity in the total dry-weight increase of the plants over a period of one to two weeks (Mitchell 1954) indicates that the quantity of carbohydrate material available over that period to the meristems was approximately the same.

REFERENCES

ABBE, E. C.; RANDOLPH, F.; EINSET, J. 1941: The Developmental Relationship between Shoot Apex and Growth Pattern of Leaf Blade in Diploid Maize. *Amer. J. Bot. 28:* 778.

AVERY, G. S. 1933: Structure and Development of the Tobacco Leaf. *Amer. J. Bot. 20:* 565.

BRIAN, P. W.; ELSON, G. W.; HEMMING, H. G.; RADLEY, M. 1954: The Plant Growth Promoting Properties of Gibberellic Acid, a Metabolic Product of the Fungus *Gibberella fujekuroi. J. Sci. Food Agric. 5:* 602.

MACDANIELS, L. H.; COWART, F. F. 1944: The Development and Structure of the Apple Leaf. *Cornell Univ. agric. Exp. Sta. Mem. 258.*

MITCHELL, K. J. 1953: Influence of Light and Temperature on the Growth of Ryegrass (*Lolium* spp.). I. Pattern of Vegetative Development. *Physiol. Plant. 6:* 21.

——————1954: Growth of Pasture Species. I. Short Rotation and Perennial Ryegrass. *N.Z.J. Sci. Tech. A36:* 193.

——————1955: Growth of Pasture Species. II. Perennial Ryegrass, Cocksfoot, and Paspalum. *Ibid. A37:* 8.

MITCHELL, K. J.; COLES, S. T. J. 1955: The Effect of Defoliation and Shading on the Growth of Short-rotation Ryegrass. *Ibid. A36:* 586.

SHARMAN, B. C. 1942: Developmental Anatomy of the Shoot of *Zea mays* L. *Ann. Bot. (Lond.) n.s. 6:* 245.

——————1943: Tannic Acid and Iron Alum with Safranin and Orange G in Studies of the Shoot Apex. *Stain Tech. 18:* 10.

——————1945: Leaf and Bud Initiation in the Gramineae. *Bot. Gaz. 107:* 269.

SOPER, KATHLEEN 1956: The Anatomy of the Vegetative Shoot of *Paspalum dilatatum* Poir. *N.Z.J. Sci. Tech. A37:* 600.

SOPER, KATHLEEN; MITCHELL, K. J. 1956: The Developmental Anatomy of Perennial Ryegrass (*Lolium perenne* L.). *Ibid. A37:* 484.

WARDLAW, C. W. 1952: Experimental and Analytical Studies of Pteridophytes. XVIII. The Nutritional Status of the Apex and Morphogenesis. *Ann. Bot. (Lond.) n.s. 16:* 207.

——————1957: Experimental and Analytical Studies of Pteridophytes. XXXV. The Effects of Direct Applications of Various Substances to the Shoot Apex of *Dryopteris austriaca* (*D. aristata*). *Ibid. 21:* 85.

A CHARACTERISTIC FILAMENTOUS BACTERIAL RUMEN ORGANISM, INDICATIVE OF A SUSTAINED LOW RUMINAL AMMONIA-NITROGEN LEVEL

By N. D. JAMIESON and T. M. LOFTUS, Animal Research Station
Department of Agriculture, Wallaceville

(*Received for publication, 11 September 1957*)

Summary

A filamentous bacterium occurring sporadically in the rumens of sheep on pasture, has been identified as a member of the family Beggiatoaceae, and shows characteristics intermediate between those of the two genera, *Thiothrix* and *Beggiatoa*.

The presence of the filaments in the rumen is indicative of a sustained level of ammonia-nitrogen, lower than that usually found in the rumen liquor of sheep on pasture. Such conditions can occur under various listed circumstances. Ammonia-nitrogen in the form of ammonium ions is not toxic to the bacterium, and its failure to develop or to be maintained in the rumen under conditions which lead to the normal moderate levels of ammonia-nitrogen may possibly be linked with the activity of ammonia-producing organisms in the rumen.

The filaments appear in samples from animals suspected of being cobalt deficient, but again the presence of a low ammonia-nitrogen level appears to be the governing factor, since it is possible to maintain the bacterium in the rumen of an animal receiving ten times its daily requirement of cobalt—provided the ammonia-nitrogen being formed in the rumen is kept at a low value.

Some preliminary attempts have been made to culture the bacterium *in vitro*.

INTRODUCTION

An organism having the form of a long segmented rod was reported by Gall as being present in the Gram-stain preparations of rumen contents of grazing sheep (Andrews *et al.* 1957). Its occurrence in the rumen flora was comparatively rare, and the examples found came from cobalt-deficient sheep.

This is apparently the same organism recorded by Hungate *et al.* (1952) as a Beggiatoa-like filament seen under the phase contrast microscope to contain granules, and present in the rumen contents of two of their sheep, and by Moir and Masson (1952) in their list of rumen organisms occurring in the sheep rumen, as Organism No. 2. "long slender Gram-negative rods composed of short uniform cylindrical cells and varying greatly in length".

This filamentous bacterium has now been found to occur in the rumen of the sheep under a number of conditions of feeding, and also in cows grazing pasture with access to hay. An attempt has been made to culture it *in vitro*, and to assess its importance in the rumen as an indicator organism—whether of cobalt deficiency, or of a sustained low ammonia-nitrogen level.

N.Z. J. agric. Res. 1: 17-30.

EXPERIMENTAL

Samples of rumen contents were obtained routinely from grazing sheep by means of a rumen tube. The fresh contents were filtered through two thicknesses of sterilized cheese cloth and the resulting rumen liquor used for observation and analysis.

Oxidation-reduction potentials and pH values were measured using a Beckman model G meter fitted with platinum and glass electrodes respectively. Ammonia-nitrogen values were determined by the Conway (1947) diffusion method. Qualitative tests for the presence of sulphydryl compounds in rumen liquor were made on aqueous dialysates from a cellophane sac, using the reaction with sodium nitroprusside in alkaline solution. The resulting colour was graded + to +++ to indicate slight, moderate, and intense reactions.

The filaments were detected in the rumen liquor by examination of a Gram stain on a film of the material. They are visible under the low-power objective (× 100); and this was used for the preliminary search of the field. Individual filaments were then examined under the oil-immersion objective (× 970). Later it was found that the filaments, when present, tended to concentrate in the froth on the surface of the sample. The following method of concentrating the filaments was used. The upper one millilitre of rumen liquor was removed from the approximately thirty millilitres of sample, shaken with four millilitres of water, stood five minutes, and a loopful of the froth taken for staining. We believe that this method makes a negative finding more reliable, and intensifies the differences between positive samples.

The degree of infestation of the rumen was assessed on the following scale:

—	Organism absent. No filaments found on the film.
+	Slight infestation. One or two filaments on the film.
++	Mild infestation. One or two filaments seen in most fields.
+++	Heavy infestation. Many filaments in nearly all fields.

Positive samples were also examined as unstained hanging-drop preparations.

RESULTS

The Occurrence of the Filamentous Bacterium in the Rumen

(a) *Sheep on Pasture and Suspected to be Cobalt Deficient.* Eleven out of fifty-two animals examined showed the presence of the filaments in the rumen. Of seventy-seven cobalt-supplemented sheep on similar pasture, only one was found to be positive.

(b) *Sheep Grazing Pasture Topdressed with Cobaltised Superphosphate.* The filaments have occasionally been found to be present in the rumen of sheep on these paddocks; this is most unusual, however, and animals carrying a heavy infestation developed elsewhere, when turned out on topdressed pasture show a degeneration and disappearance of the rumen organism in about eight days.

(c) *Transient Appearance of the Bacterium in the Developing Rumen of the Unweaned Lamb.* Five out of six lambs running with the ewes on pasture topdressed with cobaltised superphosphate showed the presence of the filaments in the rumen at the age of nineteen days; at twenty-six days of age and older, all the lambs were

TABLE 1. OBSERVATIONS ON GRAZING ANIMALS

	Degree of Infestation of the Rumen	Rumen Liquor Ammonia-N (mg/100 ml)	Rumen Liquor pH	Rumen Liquor Eh	Rumen Qualitative Test for-SH
Sheep (2)—On cobalt topdressed ryegrass clover.	+	21	6.6	—285	
,,	+ to ++	12	6.75	—300	
Unweaned Lambs— 19 days old 5 of 6 examined	+	10–16	6.95	—160	
24 days old 4 of 8 examined	+	13–20	7.2		
44 days old 6 of 6 examined	—	10–16	7.5		
Sheep— Adults at same time as above	—	30–40	6.0		
Sheep—on chou-moellier					
35 days (eating leaf)	—	21	6.7	—275	+
43 days (eating leaf and stems)	—	16	6.9	—300	++
55 days (eating stems and flowers)	+ to +++	12.5	6.9	—310	+++
3 out of 5 sheep developed an infestation					
Sheep—On swede tops	+ to ++	25	7.05	—360	
5 out of 6 sheep developed an infestation	+++	18	7.10	—375	
Cows (2)—On pasture with access to hay	+ to ++	6.5–15	7.65	—160 to —255	

negative. In another season, four out of eight lambs twenty-four days old were positive, while adult animals on the same pasture were negative. At these stages, the rumen contents of the lambs were scanty in volume, occasionally contained milk and wool residues, and showed a low level of ammonia-nitrogen—about 10.0 to 19.0 mg N/100 ml (Table 1).

In our experience, normal values for ammonia-nitrogen in the rumen-liquor samples from sheep on pasture fall within the extreme range of 10 to 75 mg/100 ml with a mean value of 30.5 mg/100 ml, the majority of the values lying between 15 and 35 mg/100 ml.

Table 1 shows that, on all occasions when the rumen sample has been positive for the filamentous organism, the corresponding ammonia-nitrogen level has been 25 mg/100 ml or lower. Although a sustained low ammonia-nitrogen level in the rumen is a necessary condition for the development of the filamentous bacterium, it is not a sufficient condition. (See also sections d and f) The filamentous bacterium detected in the rumens of 19-day-old lambs had disappeared by the time they were 44 days old, while the rumen ammonia-nitrogen values were still at the low levels of 10–16 mg/100 ml rumen liquor. The predominating large forms of organism showing in the Gram stain at this stage were rods and cocci.

(d) *Sheep Grazing Chou Moellier.* Two sheep out of three sampled after 55 days grazing chou moellier showed the presence of the filaments in the rumen in moderate to heavy concentration. A mild infestation was shown by one of two animals which had been on the fodder for the terminal 20-day period. The results are summarised in Table 1.

A similar experiment the next year failed to produce any sign of an infestation of the rumen. A sustained low ammonia-nitrogen level was not obtained; values fell towards the end of the 70-day feeding period, but were fluctuating markedly owing to the consumption of grass and weed which was beginning to take control of the paddock. The cobalt content of chou moellier stems was 0.05 p.p.m. on dry matter—low compared with normal pasture.

(e) *Sheep Fed Freshly Cut Swede Tops.* After eleven days, five out of six sheep had become positive. Two animals were only slightly, one mildly, and two moderately heavily infested. From the eleventh to the twentieth days of this regimen the ruminal infestation continued and the filaments increased in numbers in two sheep. On turning out to pasture, five positive animals became negative after one day. From the second to sixth days, sporadic positives were obtained in two animals. All were negative at seven days and subsequently. The cobalt content of the swede tops was low (0.03 p.p.m. on the dry matter).

(f) *Sheep Stall-fed on Meadow Hay and Crushed Barley.* In an experiment designed to test the hypothesis that the bacterium develops in the rumen as a result of a sustained low level of

TABLE 2. PEN-FEEDING EXPERIMENTS

Days on Ration	Treatment	Degree of Infestation of the Rumen			Rumen Liquor Ammonia-N mg/100 ml (Mean Values)		
0	Three sheep fed in pens equal parts of meadow hay and crushed barley; 360 g twice daily. Cobalt content—0.08 p.p.m.	—	—	—	—		
3		—	—	—	9.0		
9		—	—	—	10.5		
11		+	—	+	9.0		
14		+++	—	—	13.0		
20		+++	+	+	—		
26		+++	+++	+	11.5		
33		+++	+++	+	—		
38		+++	+++	—	—		
0	Three other sheep fed in pens, on the above diet.	—	—	—	(Individual values)		
20	Each sheep dosed at 11.30 a.m. with 12 g ammonium acetate in 1 litre of water. Sampled again 4.00 p.m. Repeat dose at 4.30 p.m. and twice daily thereafter.	+++	++	+++			
		+++	++	+++			
22	Sampled before morning dose	++	+	++	11.5	19	22
	,, 10 mins. after dose				65	96	56
	,, 50 mins. ,, ,,	++	+	++	57	94	50
	,, 120 mins. ,, ,,	++	+	++	41	82	38
	,, 420 mins. ,, ,,	++	+	++	11.5	32	18
24		+++	+	+++			
27	Sampled before dosing. Dose changed to 8 g ammonium chloride in 1 litre of water twice daily.	++	+	++			
28		+++	+	++			
30		+	+	++			
31	Returned to pasture after sampling and a.m. dose (Final dose)	++	+	+++			
Days on Pasture							
0	2 Sheep—From above ration and dosing.	++		+++			
3		+		++			
5		—		+			
6		+		+	13.5	12.5	
7		+		+	20.5	15.0	
12		—		+			
24							

ammonia-nitrogen, sheep were pen-fed twice daily 360 g of a mixture of equal weights of meadow hay and crushed barley. The ammonia-nitrogen values for the rumen liquor under these conditions ranged from 3.9 to 20 mg% ammonia-nitrogen with mean values of 9 to 15 mg%. Three sheep developed the bacterium in the rumen in 11 days. Six sheep, three of which were dosed twice daily with cobalt sulphate (containing 0.5 mg Co to supply about ten times the normal daily requirement, showed the filaments in the rumen by the sixth day and continued to do so for a further fourteen days, with an occasional negative in individual animals (Table 3).

TABLE 3. Cobalt Dosing during Pen-feeding

Six sheep were fed a hay plus crushed barley ration. Three of them were dosed twice daily with 0.5 mg cobalt as cobalt sulphate solution

Control Group (Sheep A, B, C.)

Days on Ration	Degree of Infestation of the Rumen			Rumen Liquor Ammonia-N. (mg/100 ml)			
	A	B	C	A	B	C	Mean
6	++	++	+	11.2	11.5	3.9	8.8
8	++	—	+	10.9	19.9	9.8	13.5
13	+++	+++	—	—	—	—	—
17	—	++	—	7.8	10.9	12.3	10.3
20	—	+++	++	—	—	—	—

Dosed Group (Sheep D, E, F.)

Days on Ration	Degree of Infestation of the Rumen			Rumen Liquor Ammonia-N. (mg/100 ml)			
	D	E	F	D	E	F	Mean
6	+	+	+	4.7	18.2	15.4	12.8
8	+	+++	++	12.9	19.3	12.6	14.9
13	+	+++	++	—	—	—	—
17	—	+	—	18.5	4.2	17.1	13.2
20	—	+	+	—	—	—	—

At the conclusion of these experiments, three animals with heavy infestations were dosed with solutions of ammonium acetate twice daily to raise the level of ruminal ammonia-nitrogen well above that normal for the hay and crushed-barley diet. As shown in Table 2, this treatment did not result in the disappearance of the bacterium from the rumen, although the ammonia-nitrogen levels showed a marked rise during the seven hours after dosing. The use of ammonium chloride in place of acetate did not alter the situation. Two of these animals were then turned out to pasture. Both showed a rapid decrease in the degree of infestation. One sheep became consistently negative after twelve days on pasture, while the other continued to show the presence of the filaments until the twenty-fourth day.

(g) *Cows on Pasture with Access to Hay.* The filaments have been observed in the rumen contents of two cows. Again the ammonia-nitrogen values for the rumen liquor were low (Fig 1b and Table 1).

Possible Infection of Clean Sheep on Pasture

Two sheep, which had been on cobalt-deficient pasture and were carrying the bacterium in their rumens, were run with clean sheep on pasture topdressed with cobaltised superphosphate. A few days later the bacterium was found to be present in three of the formerly clean sheep and persisted in one of them after the filaments had died out in the two introduced sheep. This probably forms an example of the mild infestations that can occur in sheep grazing topdressed pasture (see section b, above) rather than the transmission of a rumen organism from one animal to another. The spores of the bacterium are probably ubiquitous, and its vegetative growth in the rumen is consequent upon the onset of suitable conditions therein. If these are present, the filaments appear, and will spontaneously disappear soon after a swing away from suitable ruminal conditions. Under such unfavourable conditions, the filaments have been observed to be attacked and digested by the small rods and cocci present in the rumen.

In an attempt to transmit the bacterium, two clean sheep on topdressed ryegrass-clover pasture were given heavy inocula of rumen contents from a sheep that had developed a heavy infestation while being fed on swede tops. The bacterium persisted in the rumen of the inoculated animals for four days and died out completely by the seventh day.

Morphology of the Filamentous Rumen Bacterium

The following description is based upon the appearance of the organism in fresh unstained preparations and in Gram-stain films of rumen liquor.

The organism occurs in the form of filaments, some of which can attain considerable length (Fig. 1f). The filaments are never branched, generally occur singly, and are not permanently coiled. The organism is non-motile, but the filaments are extremely pliable and are readily carried by currents in the surrounding fluid through tortuous channels. Each filament shows transverse divisions throughout its length into fairly uniform rectangular cells, with rounded terminal cells. Granules are frequently seen in individual cells and when these are profuse they tend to obscure the division into segments (Fig. 1a).

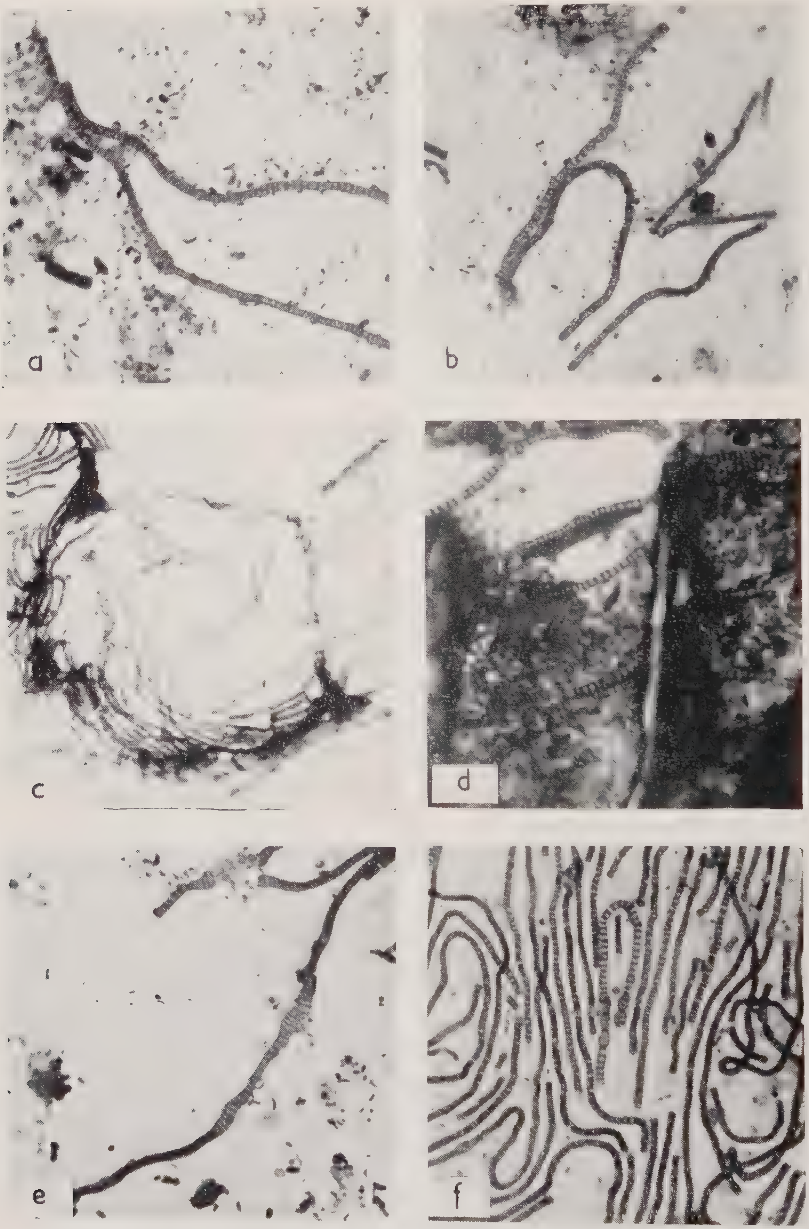


Fig. 1.—A filamentous bacterium occurring in the rumen under certain conditions. Gram's stain.

a.—Sheep on pasture. × 1,000.

b.—Cow on pasture with access to hay. × 1,000.

c.—Sheep fed swede tops. Showing the filaments on the cortical cell wall residues. × 500.

d.—Sheep fed chou moellier. Showing filaments in and on partially digested epidermal cells. × 1,000.

e.—Abnormal form, showing swelling of some of the cells of the filaments. × 1,000.

f.—Sheep fed swede tops. Washed concentrated suspension of the organism, showing spore formation. × 1,000.

Growth of the filaments in the rumen occurs by cell division and longitudinal extension of the cells. Multiplication of filaments occurs by the formation of a break, without the cells on either side of this necessarily showing any swelling. Groups of cells within a filament frequently swell with the formation of conidia or spores, and breaks can occur at these points (Fig. 1f). The impression is that this occurs in the more mature filaments or in those whose environment is becoming unfavourable.

The filaments in rumen liquor give a Gram-negative stain reaction, indicative of the healthy vegetative form of the organism. Older larger-diameter filaments and obviously degenerating forms show varying degrees of vacuolation and tend to stain Gram-positive.

If a sample of rumen liquor is stood at room temperature for some hours, some cells in the filaments swell and lose their normal appearance (Fig. 1e). This is apparently caused by abnormal localised osmotic effects in the medium.

Measurements of the cell size in sample filaments of the organism gave the following results:—

Mean cell width	1.06μ	Range	$0.91–1.27\mu$
Mean cell length	1.07μ	Range	$0.76–1.35\mu$

Although the above description of the organism as it occurs in the rumen conforms to the description given by Breed *et al.* (1948) for a member of the family Beggiatoaceae, it shows characteristics intermediate between those of the genera *Thiothrix* and *Beggiatoa.*

The filmentous rumen organism differs from *Thiothrix* and resembles *Beggiatoa* in that it has no holdfast, and the filaments show no differentiation into base and tip. It resembles *Thiothrix* and differs from *Beggiatoa* in the non-motility of the filaments and in the formation of specialized reproductive structures. Conidia and possibly swarm cells have been observed, but we have been unable to detect motility in the presumed swarm cells, owing to the uncertainty of identifying the objects seen in the rumen liquor as having been derived from the filamentous bacterium.

A preparation of *Beggiatoa* sp. from a sewage culture showed a close morphological resemblance to the filaments from the rumen, and to the light and electron photomicrographs of Johnson and Baker (1947). The sewage organism exhibited a very definite motility.

The bacterial filaments found in the rumen occur floating freely in the rumen liquor, but their most active growth appears to occur in intimate contact with the ruptured plant cells particularly cortical and parenchymal tissues (Fig. 1c, d). When ruminal digestion of the plant cells is complete, the filaments are released into the rumen fluid.

Search for Possible Extra-ruminal Sources of the Organism

In view of the widespread distribution of members of the Beggiatoaceae, a search was made for extra-ruminal sources of the vegetative form of the organism occurring in the rumen. Examinations were made of washings and macerates of pasture grass, clover, swedes, chou moellier, and cabbage. In only one instance was a filament of an apparently identical organism seen in a preparation from cabbage stem. The ruminal organism has not been found in the abomasum, even of an animal with a ruminal infestation at the time of post-mortem. The faeces of infected animals were also negative. Paddock water-troughs showed the presence of many filamentous bacterial and algal organisms, one or two of which showed a close resemblance to the rumen organism.

The demonstration of the possible extra-ruminal occurrence and distribution of spores of the rumen bacterium would depend on the use of an adequate method of *in vitro* culture which is not yet available.

Biochemistry of the Filamentous Rumen Organism, and Attempts to Culture it in vitro.

Attempts to culture the filamentous bacterium became worthwhile only when animals showing a heavy infestation were available (Fig. 1c). In these, the filaments tended to be trapped in the foam on the surface of the sample, and by removing this and washing it three to four times with carbon-dioxide-gassed artificial saliva (McDougall, 1948), it was possible to obtain a concentrated suspension of filaments suitable for use as an inoculum. This material still carried other rumen organisms as contaminants (Fig. 1f).

The filaments failed to grow, and degenerated rapidly when inoculated on various standard media and incubated aerobically and anaerobically. Czapek-Dox agar was the most promising of those tried, and modified media were based on this. The source of carbon appeared to be important. The use of glucose, saccharose, and other sugars, favoured the development of contaminants, which rapidly overgrew the filaments and attacked them, or flooded the medium with unfavourable metabolic products. It was found that several organic acids commonly occurring in plants supported a slow growth of the required organism; these included malate, fumarate, and tartrate.

The most successful medium used so far has the following composition:

Sodium potassium tartrate	10.0 g
Sodium hydrogen malate	5.0 g
Sodium fumarate	5.0 g
Fructose	10.0 g
Sodium nitrate	3.0 g
Potassium dihydrogen phosphate	1.0 g
Dipotassium hydrogen phosphate	1.0 g

The above was made up to 500 ml with distilled water and sterilized at 15 lb for 20 min, pH 4.8.

Sterilized artificial saliva (McDougall, 1948) 500 ml, containing 3 ml of added thiol-acetic acid, was gassed with carbon dioxide to pH 6.9.

The two solutions were mixed to give the final medium; pH 6.5, E_h—250 mV (relative to the calomel electrode).

All incubations using this medium were done under carbon dioxide at 37°C in a McIntosh-Fildes jar.

Although satisfactory slow growth of the rumen organism has been maintained for periods up to thirty days, we have not attained the optimal conditions that can on occasion appear *in vivo* and result in a heavy ruminal infestation appearing within about seven days.

It must be emphasised that pure cultures have not be obtained, and any conclusions concerning the biochemistry of the organism are likely to be invalidated by this.

The main problem is to obtain a sufficiently pure inoculum. There is some evidence that brief exposure to acid conditions at pH 4.5 kills off many of the other rumen organisms normally present. The required organism persisted well in a medium containing hydroxylamine hydrochloride, and also in one containing sodium glyoxal bisulphite where, as well as forming a source of sulphur, the sulphite presumably acted as a sterilizing agent against contaminants.

Some of the conditions favouring growth of the organism *in vivo* are given in Table 1. Rumen pH and levels of total volatile fatty acid are normal. The rumen organism is probably a sulphur bacterium, the only evidence for this being the appearance in the filaments of granules extractable with carbon disulphide. Attempts to improve the culture media by the addition of various sulphides and sulphur compounds failed. The thio-acetic acid used may supply sulphur, but any benefit it confers could be solely due to its influence on the oxidation-reduction potential of the medium. Note the rise in sulphydryl content of the rumen liquor for sheep on chou moellier.

Growth of the filaments was better under carbon dioxide than under nitrogen or hydrogen. It is unlikely that the bacterium is a strict anaerobe. In view of the organic acid substrate used in the medium, and the fact that the culture was not pure, the bacterium may be able to utilise carbon dioxide as a source of carbon. However, an attempt to culture it in an inorganic medium containing only artificial saliva under carbon dioxide was not successful. The sustained low ammonia-nitrogen criterion established for *in vivo* growth is confirmed by the deleterious effect on the *in vitro* growth of the filaments of media, natural or synthetic, high in protein and amino-nitrogen. Apparently the bacterium is capable of utilising nitrate as a source of nitrogen.

Any attempt to increase growth in the cultures by the addition of yeast salts or single members of the B-vitamin complex merely resulted in an overwhelming growth of contaminants.

Under the conditions of culture, the growth of very small-diameter filaments was observed, as well as filaments of normal size. The filaments *in vitro* also showed a tendency to undergo fission into clusters of two-cell units. These have occasionally been observed in rumen samples. An acid pH produces a characteristic effect on the cells of the filaments, from which they can recover, consisting of a retraction of stainable cell contents along the filaments, with the appearance of non-staining gaps between.

Conditions which favour the growth of the filaments, not including the low pH, also appeared to favour the growth of the ruminal sarcinae and selenomonads carried over in the inoculum.

Further refinements in cultural technique are dependent on improved methods of preparing an enriched inoculum free from other contaminating rumen organisms, and in improving the medium to give more rapid growth.

Discussion

The filamentous bacterium under consideration affords an example of the occurrence in the rumen of a member of a group of bacteria whose occurrence is widespread, and whose nutritional requirements are comparatively simple. Our interest stems from the possible association of the organism with sheep in a state of cobalt deficiency. If this were so, the readily detectable filaments would have formed an extremely valuable indicator of the condition.

The only consistent chemical findings on the rumen liquor of animals exhibiting the organism have been the low values for ammonia-nitrogen. The sustained low ammonia-nitrogen level prevailing in the rumen liquor has been shown to be the governing factor in the development of the filaments in the rumen, and may depend to a great extent on the feeding habits of the particular animal involved (together with the presence and activity of the ammonia-producing organisms in the rumen). A sustained low ammonia-nitrogen level in the rumen is not necessarily harmful to the animal, in that it may be merely reflecting a very efficient utilization of nitrogen by the rumen population as a whole. The artificial method used to raise the level of ammonia-nitrogen, dosing animals with ammonium salts, showed that the ammonium ion *per se* is not toxic to the bacterium. Under natural conditions, however, elevation of the level of the ruminal ammonia-nitrogen would be brought about by the activities of the de-aminating and ammonia-producing organisms of the rumen. It may well be that these organisms are responsible for the disappearance of the filaments under normal pasture conditions. The appearance of the bacterium in the rumen under conditions of sustained low ammonia-nitrogen would then be a reflection of the lowered activity of these ammonia-producing organisms. In our experience there have been no untoward effects shown in sheep on any of the feeding experiments to lower the rumen ammonia-nitrogen and bring on an infestation of the bacterium nor any noticeable loss in condition on the part of the animals concerned. The filaments do not appear when an animal is fasted for twenty-four hours, a procedure which would temporarily bring the ruminal ammonia-nitrogen down to an appropriately low level, although a fast or falling off in appetite does enhance the apparent degree of an existing infestation.

The culture experiments indicate that the bacterium is capable of utilising nitrate as a source of nitrogen, and of withstanding a low pH such as might obtain in the cell sap of many plants. Direct observation has shown that the filaments can grow in and on ruptured plant cells and only become free in the rumen when the plant residues are digested away. It may be that the acidity of the cell sap in the ruptured plant material affords protection from immediate invasion on the part of the normal rumen organisms. The filamentous bacterium apparently thrives in isolated pockets, under conditions abnormal to the general milieu of the rumen, and when it encounters normal conditions, undergoes rapid parasitisation and dissolution by the small rods and cocci normally present in large numbers.

Concerning the possible association of the filamentous rumen organism with cobalt deficiency; the filaments have not been observed to be present in the rumens of all sheep believed to be cobalt deficient or on the margin of deficiency, while they have been found occasionally in sheep which were presumably cobalt sufficient, sporadic appearances of the filaments having been observed in sheep grazing pasture topdressed with cobaltised superphosphate. The fact that the bacterium can appear transiently in the rumen of the young unweaned lamb, on cobalt-topdressed pasture, does not appear to be compatible with cobalt deficiency. Finally, the fact that it is possible to bring on an infestation in an animal that is being dosed with ten times the estimated daily requirement of cobalt, and to maintain it in the face of continued cobalt dosing, would indicate that there is no direct connection between cobalt deficiency and the development of the filamentous bacterium in the rumen. Its association would seem to be valid only insofar as the condition of cobalt deficiency might conceivably tend to lead to prolonged low ammonia-nitrogen levels, presumably as a result of a lowered activity on the part of the ammonia-producing organisms of the rumen. This possibility is to be investigated.

ACKNOWLEDGEMENTS

We are indebted to Mr. I. G. McIntosh and Mr. E. D. Andrews of this Station for the use of some of their experimental animals, and to Mr. A. L. Bryant for the photomicrographs.

REFERENCES

ANDREWS, E. D.; GALL, L. S.; HART, L. I.; ANDERSON, J. P. 1957: Effects of Orally Administered Penicillin on Cobalt-deficient and Cobalt-dosed Lambs. *N.Z. J. Sci. Tech. A38:* 654-63.

BREED, R. S.; MURRAY, E. G. D.; HITCHENS, A. R. 1948: "Bergey's Manual of Determinative Bacteriology". 6th ed., pp. 988-93, Williams and Wilkins, Baltimore.

CONWAY, E. J. 1947: "Microdiffusion Analysis and Volumetric Error". Rev. ed. pp. 98-113. Crosby Lockwood, London.

HUNGATE, R. E.; DOUGHERTY, R. W.; BRYANT, M. P.; CELLO, R. M. 1952: Microbiological and Physiological Changes Associated with Acute Indigestion in Sheep. *Cornell Vet. 42:* 423-9.

JOHNSON, F. H.; BAKER, R. F. 1947: The Electron and Light Microscopy of Beggiatoa. *J. Cell. Comp. Physiol. 30:* 131-45.

MCDOUGALL, E. I. 1948: Studies on Ruminant Saliva. 1. The Composition and Output of Sheep's Saliva. *Biochem. J. 43:* 99-109.

MOIR, R. J.; MASSON, M. J. 1952: An Illustrated Scheme for the Microscopic Identification of the Rumen Micro-organisms of Sheep. *J. Path Bact. 64:* 343-50.

RESISTANCE TO YELLOW-LEAF DISEASE IN *Phormium tenax* Forst. AND THE OCCURRENCE OF DELAYED EXPRESSION OF SYMPTOMS

By W. R. Boyce, Botany Division, Department of Scientific and Industrial Research, Christchurch*

(*Received for publication, 18 October 1957*)

Summary

One cultivar and two seedling progenies from cultivars of *Phormium tenax* have been tested experimentally for resistance to transmission of yellow-leaf disease by *Oliarus atkinsoni* Myers. Results show that cultivar 'SS' is resistant to but not immune from the disease, that second inbred generation (S_2) seedling progeny of '301' is highly susceptible, and that first inbred generation (S_1) progeny of 'SS' is intermediate, that is, less resistant than 'SS' but not as susceptible as '301' S_2. Evidence of long delay in the expression of symptoms was obtained during the course of this experiment and from observations continued after the conclusion of formal trial work.

Introduction

The discovery of a vector of yellow-leaf disease of phormium, the plant hopper *Oliarus atkinsoni* (Boyce *et al.* 1951; Cumber 1953), has provided a means of testing for resistance to the disease under controlled conditions. The purposes of this trial were to compare the resistance of cultivars 'SS', 'SS' S_1, and '301' S_2 to insect transmission of yellow-leaf disease by *O. atkinsoni,* and to determine whether the resistance of 'SS' was maintained against controlled test feeding.

Field observations had shown previously that 'SS' is usually highly resistant to the disease, and that '301' seedling progenies were highly susceptible. No definite conclusions concerning the resistance of 'SS' seedling progenies had been drawn from similar observations. All stocks of the original cultivar '301' had been lost through its high susceptibility to yellow-leaf disease. Plants were therefore not available for use in this trial.

Seedling progenies of '301' had previously shown susceptibility to test feeding by *O. atkinsoni.* (Cumber 1953; Boyce *et al.* 1953).

Methods

From transmission trials carried out December–January 1950–51, information was available on the percentage of infections that could be obtained by using adult *O. atkinsoni* collected in the field from diseased *P. tenax* bushes. (Cumber 1953; Boyce *et al.* 1953). For the purposes of this trial it was estimated that by using similar means 50% of test plants could be infected, and that with this percentage of infections 40 test plants from each of the three lots to be compared would be sufficient to show a 16% difference in susceptibility (A. C. Glenday, pers. comm.).

---

* Now Horticulture Division, Department of Agriculture, Levin.

Fifty-one test plants and 10 controls were used in each of the three lots under test.

Plants for use in this experiment were:

1. 15-month-old seedlings of 'SS' S_1 and of '301' S_2 taken in 1952 from open seed beds sown in June 1950 in a nursery at Paiaka*. To reduce the chances of including stray field-infected plants, only plants in the centre rows of beds were lifted, on the assumption that infections were more likely to occur on plants of outer rows. No infection appeared in check plants of 'SS' S_1 and '301' S_2 so taken.
2. Vegetatively produced plants of 'SS', of similar size to the seedlings mentioned above, were taken from propagation beds in Paiaka nursery. (These 'SS' might be regarded as clonal material, but are not so termed as the exact origin of this vegetatively multiplied stock is not known.)

Plants were trimmed to a height of 6–8 in. and potted in 6-in. pots on 30 October 1952. Pots were placed immediately in a glasshouse screened to prevent the entry of stray *Oliarus*.

In preparation for caging insects on the test plants, sleeves approximately 13 by 5 in. were made from muslin with 24 meshes to the inch. These sleeves were put over the plants and fastened with rubber bands to plugs of cotton wool placed round the bases. The tops were closed by folding them over and securing the folds with rubber bands. Insects were introduced by opening the top, inserting the mouth of a test tube in which insects had been collected (see below), shaking them in, and closing.

This method of caging worked satisfactorily except where rapidly growing leaves penetrated the weave, and the fold of the leaves could then provide a channel of escape for the insects. This was prevented by frequent inspection and folding over of protruding leaf tips within the cages.

Collections of insects were made almost entirely from old experimental plots of cultivar '273' with a few collections taken from adjoining plots of cultivar 'Ngaro' at Paiaka. All plants remaining in these plots were diseased and would have been so for the two preceding years, during which time recently emerged adults would have fed as nymphs on diseased plants. This assumes that most of the insects collected emerged on bushes within these plots, although the possibility that a number of insects could have flown in from further afield is not discounted.

Insects were collected directly into 6 x $\frac{3}{4}$ in. test tubes, which, in spite of a few losses in the course of capturing the hoppers, proved to be the most rapid method where insects were plentiful. Ten insects were collected in each tube ready for application to the test plants. Where insects were present in high numbers, a collection rate of 250 per hour per collector was attained, with a maximum rate of 400 per hour under very favourable conditions. The insects were taken directly from the field and applied to test plants as soon after collection as possible. It was necessary to apply insects to different replications on successive days as sufficient insects for the whole trial could not always be collected and applied in one day.

---

* Paiaka nursery was situated on the north bank of the Manawatu River, in the Moutoa district, ten miles from Foxton.

The first application of insects, 10 per test plant, was carried out on 5 and 8 December 1952. The second application was made on 15 and 16 December. Insects from both of these applications were left in the cages until the cages were removed on 14 January 1953, by which date only a few lethargic hoppers remained alive. As the cages were removed, these were killed to prevent stray infections, and the cages were left on the control plants longer as a precaution.

Control plants and test plants from each cultivar were randomised in position in three replications on the glasshouse benches. Plants in this trial were first examined visually for signs of disease fifteen weeks after the application of insects. A final examination was made 48 weeks after the application of insects and at this time if the condition of a plant was doubtful roots were inspected, and the rhizome was sectioned for microscopic examination after staining with phloroglucinol and acid.

After the final examination of this trial at Moutoa in November, 1953, all plants were lifted from pots, and from those that remained intact 40 test plants of 'SS' and 26 test plants of 'SS' S_1, which appeared to have resisted infection, were transferred to Botany Division's area at Bryndwr, Christchurch, for further observation. Here, in a district where *O. atkinsoni* is not known to occur, the plants were replanted in December 1953 in open ground. It was not possible to maintain the original form of the trial or to provide adequate control plants after the move from Moutoa.

Most of the transplants established and made growth during the summer and autumn. In August 1954 these plants were re-examined. It was apparent then that symptoms of yellow leaf were still developing and these could not readily be attributed to causes other than the deliberate insect inoculations made more than two years previously, although in the absence of control plants after the transfer to Christchurch it is not fully proven. The condition of the plants was recorded for the last time in April 1955.

RESULTS

Survival of insects in the cages was good. They fed actively for several days after application to the plants, and many from the first application still survived when the second batch of insects was applied. The plants did not appear to suffer any serious damage under the cages, although populations of red spider-mite had built up on some plants and had caused a superficial chlorosis of the leaves.

TABLE 1. CONDITION OF PLANTS AT 15 WEEKS AND 48 WEEKS AFTER TEST FEEDING.

	Cultivar	Condition of Test Plants all Replications				*Percentage of Healthy Test Plants	Condition of Control Plants all Replications				Analysis of Results		
		H	D	?	X		H	D	?	X		χ^2	P
15 weeks	$SS.S_1$	46	4	0	1	92.0 ± 3.8	10	0	0	0	$SS.S_1$ v. $301.S_2$	58.8	<0.001
	$301.S_2$	10	39	2	0	20.4 ± 5.8	10	0	0	0	$SS.S_1$ v. SS	0.37	0.60
	SS.	44	7	0	0	86.3 ± 4.8	10	0	0	0	$301.S_2$ v. SS	41.0	<0.001
48 weeks	$SS.S_1$	29	21	0	1	58.0 ± 7.0	10	0	0	0	$SS.S_1$ v. $301.S_2$	24.28	<0.001
	$301.S_2$	5	46	0	0	9.8 ± 4.2	10	0	0	0	$SS.S_1$ v. SS	9.18	0.001
	SS.	42	6	1	2	87.5 ± 4.7	9	0	0	1	$301.S_2$ v. SS	56.71	<0.001

H = Healthy.
D = Diseased.
? = Diseased, origin of disease uncertain.
X = Dead or missing.
* Expressed as percentage of H + D plants only.

The condition of all plants at the first and final examinations carried out during the course of the trial at Moutoa is given in Table 1.

In the first examination 'SS' and 'SS' S_1, showed almost equally high resistance to infection judged by the number of plants remaining healthy. At the second examination there had been a marked reduction in the number of 'SS' S_1 plants remaining healthy, from 92% to 58%, and a further reduction in '301' S_2 from 20% to 9.8% of test plants remaining healthy. On the other hand the number of healthy 'SS' plants has been reduced by only two plants.

The case history of plants transferred to Christchurch is shown in Table 2 below. These final observations were made in April 1955, 18 months after the last examination recorded in Table 1, and 28 months after insects were caged on the plants.

TABLE 2. CONDITION OF PLANTS 28 MONTHS AFTER TEST FEEDING

	Number of Test Plants Transferred November 1953	Condition of Plants Examined April 1955			
		Percentage			
	Apparently Healthy	App. Healthy	*Sub. norm.	Diseased	Dead
SS	40	62.5	17.0	17.5	2.5
'SS' S_1	26	20.6	8.8	38.2	32.3

*Plants lacking in vigour or showing an unusual growth form but not otherwise exhibiting symptoms of yellow-leaf disease.

Five plants of 'SS' and seven plants of 'SS' S_1 not noted as diseased plants in November 1954, 12 months after transfer to Christchurch, were recorded as diseased in April 1955.

DISCUSSION

A comparison has been made under controlled experimental conditions of the reactions of a cultivar, and of two seedling progenies of cultivars, of *P. tenax,* to insect-transmitted yellow-leaf disease.

There are marked differences in susceptibility and in the rate of expression of symptoms. Delay in the expression of symptoms appears to be associated with resistance, but the nature of this relationship is not known.

There is well supported evidence from this trial that symptoms of yellow-leaf disease may not appear in some plants until twelve months after inoculation. There is strong presumptive evidence that the delay may be as long as 28 months. Chamberlain (Boyce *et al.* 1953) has previously reported an equally long delay in the expression of symptoms in a case of graft transmission where older plants were used.

The method used for inoculating plants in this trial depends for its application on the ready availability from the field of large numbers of infective insects. More information is required on the characteristics of the disease before similar methods of testing could be used with confidence for the selection of dependably resistant plants in a resistance breeding programme.

CONCLUSION

Of the original number of test plants inoculated in this trial approximately 50% of 'SS' and 14% of 'SS' S_1 remained apparently healthy 28 months later.

The value of the cultivar 'SS' is well established by its persistence and productivity in plantations where more susceptible plants have failed. But these results should emphasise the fact that it is not immune, although it possesses a useful degree of resistance. The possibility must be considered that this resistance takes the form of a partial tolerance to the disease. For this reason 'SS' stock should not be distributed from disease-infested areas to localities where, in the presence of a vector, it might act as a source of infection.

The ultimately low level of resistance shown by 'SS' S_1 renders its usefulness as a disease-resistant line for commercial production doubtful.

Clonally propagated stock of the cultivar 'SS' is therefore the more dependable source of phormium resistant to yellow leaf. But, as it is not immune from the disease, its use in commercial planting should be accompanied by the development of cultural methods (e.g. controlled flooding) designed to prevent the build up of insect populations and an incidence of infections which might lead to a breakdown of resistance.

ACKNOWLEDGEMENTS

Dr. R. A. Cumber, Entomological Research Station, gave advice and assistance on the collection and the handling of insects.

Dr. R. V. Mirams and Mr. J. A. Swindlehurst assisted in setting up the experiment, and with the collection and the application of insects. Mr. Swindlehurst also assisted with the examination of plants for symptoms of disease.

Mr. A. C. Glenday, Applied Mathematics Laboratory, gave advice on the design of the experiment and analysed the results shown in Table 1.

REFERENCES

BOYCE, S. W.; BOYCE, W. R.; CHAMBERLAIN, E. E.; CUMBER, R. A.; FRY, P. R.; MATTHEWS, R. E. F.; NEWHOOK, F. J.; STRZEMIENSKI, K. 1951: Preliminary Note on Yellow-leaf Disease of *Phormium. N.Z.J. Sci. Tech. A.33:* 76–7.

BOYCE, S. W.; BOYCE, W. R.; CHAMBERLAIN, E. E.; FRY, P. R.; MATTHEWS, R. E. F.; NEWHOOK, F. J. 1953: Investigations into Yellow-leaf Disease of *Phormium.* V. Transmission Experiments and Discussion on Control *N.Z.J. Sci. Tech. A.34 (Suppl. 1)*: 41–6.

CUMBER, R. A. 1953: Investigations into Yellow-leaf Disease of *Phormium.* IV. Experimental Induction of Yellow-leaf Condition in *Phormium tenax* Forst., by the Insect Vector *Oliarus atkinsoni* Myers (Hem., Cixiidae) *N.Z.J. Sci. Tech. A34 (Suppl. 1)*: 34–40.

EFFECT OF "METASYSTOX" ON THE CABBAGE APHID (*Brevicoryne brassicae* (L.))

By A. D. Lowe, Entomology Division, Department of Scientific and Industrial Research, Ashburton

(*Received for publication, 7 October 1957*)

Summary

In an experiment by Palmer-Jones *et al.* (1957) to test the effect of "Metasystox" on bees when applied to a crop of chou moellier (*Brassica oleracea* L.) in full bloom, opportunity was taken to assess the effects on the cabbage aphid (*Brevicoryne brassicae* (L.)) and its predator-parasite complex, and to make what preliminary observations were possible on the effect of this material on humans working in the crop. Counts taken showed that complete control of aphids took place, and that this condition was maintained until near harvest, a period of approximately 50 days. Little detrimental effect on parasites or predators was recorded. Humans suffered no drastic symptoms in the face of limited deliberate exposure.

Introduction

During the spring of 1956, the Animal Research Station of the New Zealand Department of Agriculture wished to estimate the effect of a new insecticide "Metasystox" on bees, by applying this material to a crop of flowering chou moellier (*Brassica oleracea* L.). This Division was invited to assist in the choice of a suitable field, and in generally organising the experiment.

The general background of the experiment, a technical description of the material used and its application, and of aphid control in cruciferous seed-crops and its relation to the conservation of bees, have been given by Palmer-Jones *et al.* (1957). It was agreed that if a suitably infested field were chosen, opportunity could be taken to assess the effects of this material on the population of the cabbage aphid *Brevicoryne brassicae* (L.) and its predator-parasite complex. Since the material was also claimed to be much safer to use than previous compounds of a similar nature, it was considered that any observations possible under field conditions, of its effects on humans, would help in the understanding of its toxicology.

Experimental Method

Layout

The design of the experiment was primarily for the purpose of studying the effect of this material on bees visiting the crop. But it was also important to assess the effect of the treatment on the aphid population since, in practice, control of these insects would be the only real reason for applying an insecticide to a cruciferous crop at any time near flowering. But an experiment designed to study the effect on bees precludes the inclusion of untreated controls, since these areas would act as an alternative source of nectar, attracting bees away from the treated crop. This would render it impossible to assess the effects of the spray on the bees working in the treated area.

It was also desired to simulate as far as possible the conditions likely to be encountered by bees under future commercial field-spraying conditions. Under such conditions, whole fields would be treated, probably leaving no alternative nectar source for bees, which would thus be compelled to continue visiting the treated crop.

For these reasons, an isolated field of crop in full bloom was chosen for this experiment, and the whole area treated. However, one or two small areas at the margins of the paddock, where spraying swaths failed to overlap, remained untreated, and these gave opportunity for some counts of aphids on untreated areas without affecting the main intention of the bee experiment. The general pattern of field build-up of populations of this aphid had been studied during the two previous seasons, and this information indicated seasonal trends against which the observations of this experiment could be checked.

Application

Twenty fluid ounces of Metasystox was applied by air in seven gallons of water per acre in the early evening of 16 October 1956. The aircraft employed was a closed-cockpit model—an Auster Aiglet. It was fitted to deliver approximately seven gallons of spray per acre at a pressure of 35 lb/sq in. using nozzles having a $\frac{1}{8}$ in. orifice and spaced at 5 in. intervals. It was considered by all observers that very good coverage was obtained, and that the operation simulated in all respects a normal well-executed commercial field-spraying. The aircraft made three circuits, delivering on each occasion a load of 25 gallons of spray material consisting of 3.5 pints of Metasystox and 24.5 gallons of water. Slight drift occurred, the wind speed varying between 5.5 and 9 m.p.h. during the 20 minutes taken by the flying operation. It was considered that not less than 16 fl oz of Metasystox per acre would reach the plants, the plane flying about 5 ft. above the crop throughout. At the conclusion of the operation the plane was surface decontaminated with a plentiful supply of water, and the spray tank was washed out and refilled with clean water which was later jettisoned over waste country. The pilot chose not to wear mask, goggles, or overalls, all of which were being supplied to all parties participating.

Aphids: Sampling

Considerable work on the sampling and counting of field populations of the cabbage aphid had been undertaken by this Division, and a project was under way to develop a method for the more rapid counting of large numbers of aphids. This was primarily designed to allow more extensive sampling and counting with the limited manpower available. Pending the full development of this technique, a method of sampling in lanes had been adopted for the study of the build-up of field populations, and this method was, with full recognition of its limitations, used in this experiment.

This method of sampling consisted in selecting a lane for each of two officers by random methods. Commencing at the boundary of the crop each officer collected 12 samples at 20-pace intervals, selecting as his sample the plant nearest to his foot at the end of each twentieth pace. Lanes were re-selected randomly at each sampling, and the use of the plant nearest the boot as the sample precluded any deliberate selection. The whole seedhead was taken as the sampling unit, and enclosed in a paper bag. In the laboratory, the aphids were killed by being washed into a funnel with hot water (*ca.* 190°F), and counted in a petri dish over squared paper, using a hand tally-counter. Weekly samples were taken throughout, commencing approximately four weeks before application. Additional daily samples were drawn immediately after the treatment was applied, and thereafter periodically until harvest. Results are given in Table 1, the figures shown each representing the arithmetical mean of 24 samples.

It has been found in practice that this method of sampling, using two officers, gives a consistent picture of the population trends within a field over the season, based on weekly counts (see Table 1), but that it is of limited value in comparing treated and untreated areas, except where large differences occur. It was considered that this method was a reasonable one to apply in studying the build-up prior to the experiment, and in assessing the control obtained, using the limited manpower available.

Parasites

Allotria brassicae Ashm. and *Aphidius rapae* Curt. are commonly found parasitising the cabbage aphid in Canterbury. To test the survival of these insects after spraying, 20 heads of chou moellier were picked at random and confined in jars in the laboratory. Numbers of parasites emerging are given under "Results".

Predators

The ladybird *Coccinella undecim-punctata* L. and the syrphid *Syrphus novae-zealandiae* Macq. were present and some counts were made to indicate survival following treatment, as set out under "Results".

Human Hazards

The systemic organo-phosphorus compounds used as insecticides are all highly toxic to mammals. Metasystox, however, is claimed to be of low toxicity to mammals, so it was agreed to try, without taking any foolish risks, to assess this factor during the experiment. Risk with this material is said to be associated only with mixing of the raw material, when the skin must be kept covered. Rubber gloves, respirators of an approved pattern, and overalls were worn by all persons involved in the mixing. The pilot took no part in the mixing and chose to work without these protective devices during actual spraying operations. All operators watched the application from a point well clear of drift.

Those taking subsequent counts, which involved daily moving through the crop for a period up to one hour for aphids, and several periods of one hour at intervals each day for bees, were advised to take full precautions against possible hazards. The two operators collecting aphid samples dispensed with respirators for about 15 minutes each day for the first 3 days after treatment, while moving through the crop. Those counting bees made counts several times daily for 9 days following treatment, and took full protective precautions. After 15 days, aphid counts were taken weekly or fortnightly involving walking through the crop for 15–20 minutes without any protective devices other than overall suits. Observations of toxic hazards are recorded under "Results".

RESULTS

Aphids

Counts of aphids taken from 14 September to the date of the experiment, 16 October 1956, and for approximately two months afterwards, are shown in Table 1, together with similar counts taken in the 1955 season when other experiments were being made. The population trends revealed in these figures should be considered in the light of the discussion given later in this paper.

TABLE 1. APHIDS PER SEED HEAD
(Arithmetical mean of 24 samples)

1955 Season		1956 Season		
21 Sept.	354	14 Sept.		67
23 Sept.	260	20 Sept.		25
27 Sept.	364	27 Sept.		102
30 Sept.	296	4 Oct.		126
7 Oct.	293	10 Oct.		165
14 Oct.	347	16 Oct.	10 a.m.	255
1 Nov.	408		7 p.m.	Treated with Metasystox
8 Nov.	601	17 Oct.	10 a.m.	Nil
15 Nov.	640	18 Oct		Nil
		19 Oct.		Nil
		24 Oct.		Nil
		31 Oct.		Nil
		14 Nov.		Nil
		28 Nov.		Nil
		13 Dec.		Nil

The count of aphids taken on 17 October on treated samples gave a total of 70 aphids which, though classified in Table 1 as dead, still showed slight signs of life when counted—16 hours after application. The vast majority of aphids were dead and had fallen from the plants. Even those on low sheltered plants had failed to survive. These 70 surviving aphids were all young nymphs, showing little sign of activity with legs doubled up. Antennae were twitching vigorously, and the stylets were still inserted and appeared in all cases to be the only reason for the attachment of the insect to the plant. They were confined in petri dishes with fresh food overnight. A control group of untreated aphids was similarly confined. Twenty hours after confinement, 100% of the treated aphids were dead, while only 6% of the control group succumbed. It is therefore considered that all aphids recorded as dead on 17 October were actually killed by the spray as indicated in Table 1 on this date.

As mentioned earlier in this paper, normal untreated control counts were not possible because of the special requirements of the apiary side of this experiment. However, one or two small areas at one end of the field missed treatment owing to failure to overlap the swath during spray operations, and these untreated areas gave useful check counts. Fifteen days after treatment, typical untreated plants (4 samples) had a mean population of 981 aphids per plant; 29 days after treatment a similar count (6 samples) averaged 193 aphids per plant. These figures should be considered in the light of the remarks about predaceous and parasitic insects, and their attention to surviving aphids, under "Discussion".

Parasites

From 20 seed-heads collected, the following parasites emerged after 2 weeks from the 35 parasitised aphids found thereon:

Allotria brassicae 6	*Aphidius rapae* 21

Predators

Ladybirds were almost entirely in the adult stage at the time of treatment, and showed no symptoms of damage. Several counts on days following treatment showed that ladybirds on the ground numbered 1 dead to every 12 alive. Syrphid adults were plentiful in the crop on the days following treatment and showed no signs of damage. On 14 November, counts taken over two 100-pace randomly selected lanes through the crop gave counts of 1 and 2 adults respectively. Two similar counts through untreated marginal areas still infested with aphids gave 12 each.

Syrphid larvae are difficult to discern in heavy aphid colonies. On 17 October only 1 larva was seen on a count of 100 heads at random, and this insect appeared to be badly affected by the treatment. By 18 October most of the dead aphids had fallen to the ground, clearly revealing the remaining syrphid larvae. A count of 100 heads at random gave 10 dead larvae and none alive.

Human Hazards

Those operators who dispensed with respirators for a period each day reported a strong acrid rather salty taste after about 15 minutes spent in the crop. This persisted overnight for as long as exposure continued. Other operators at first discounted any suggestion of contamination, but after several days agreed that they too experienced the salty taste persisting overnight. No actual toxic symptoms of the type usually associated with organo-phosphorus materials were experienced by any of the ground operators.

The pilot reported the next day that he felt no ill effects but later this report was changed and qualified. This observer's statements must therefore be regarded as inconclusive.

It should be noted from Palmer-Jones' (1957) record of the conditions at the time, that weather during these observations was fair and warm, with shade temperatures on several occasions exceeding 65°F.

Economics

It is appropriate that some mention should be made of the economic results of the treatment given to this crop. The cost of the materials used was approximately £1/5/- per acre. There is evidence that in the absence of some curative treatment the crop would have been ruined by the aphid infestation. Under the conditions of this experiment, the yield was 430 lb of seed, valued at £65, per acre. The possibility that a high population of bees increased pollination, and thus seed-setting, should not be overlooked in this connection.

DISCUSSION

Previous studies of the build-up of this aphid in chou moellier, following the overwintering of apterous females in sheltered parts of the plant, had shown that there was an increase in numbers as the spring period progressed, parasites and predators exercising little control until late December. It is considered that the 1955 figures are typical of the seasonal trend, and that the 1956 figures, in Table 1, taken in conjunction with the mean count of 981 aphids per plant on an untreated area on 31 October 1956, show that the 1956 season followed the usual trend.

The heavy reduction in untreated counts by 14 November 1956 should be considered in the light of observations recorded on predacious and parasitic insects, and the attention they subsequently gave to the small areas that had escaped treatment. Those familar with insect habits will realise that surviving predators and parasites would quickly bring their attention to bear on any remaining aphids and reduce their numbers.

The isolated nature of the field gave little opportunity of reinfestation by the cabbage aphid, and the nil counts recorded following treatment should be considered accordingly. Observations in untreated crops in both the 1955 and 1956 seasons indicate that general re-distribution of the aphid population takes place in spring flights, which occur in Canterbury during the last few days of October. The "nil" counts after this date are probably due to the isolated nature of the field rather than to any prolonged action of the treatment given.

There is no obvious explanation of the fact that all aphids surviving on 17 October were young nymphs, although it is possible that they had consumed insufficient contaminated food to cause early mortality. It appears likely, however, that, either by contact or feeding, Metasystox was finally the cause of death. Otherwise, a further build-up from this residue of very young insects would have been noted in the field.

Observations on human hazards are difficult both to record and evaluate, but it should be noted that all the ground participants were experienced and reasonably well-informed operators. One of the difficult aspects of evaluation is in trying to differentiate betwen the taste of the insecticide and the strong acrid flavour of pollen, which is fairly heavily borne in flowering crucifers.

CONCLUSIONS

Control of Aphids

Though the absence of control counts limits the conclusions that may be drawn in an experiment of this kind, it is considered that it is beyond any reasonable doubt that, under the conditions of this experiment, infested plants were cleared of all stages of the cabbage aphid by the use of Metasystox as an aerial spray, and that there is some evidence that without some such treatment the infestation would have reached very heavy proportions, and probably ruined the seed-crop, resulting in severe financial loss to the grower. The isolated nature of the crop apparently prevented reinfestation, and it may be concluded that once an isolated field has been freed of its primary spring build-up, which in turn is a result of survival of the over-wintering stages, it could remain free from aphids until it is harvested.

Parasites and Predators

Metasystox appeared to do little damage to the parasites and predators present, except in the case of larval Syrphidae, which were killed by direct exposure to the spray.

Human Hazards

The generally agreed opinion among operators as to the persistent nature of the acrid taste experienced after treatment, leads to the conclusion that continued exposure gives rise to some absorption of Metasystox, but without the production of the drastic symptoms normally associated with absorption of organo-phosphorus materials used as insecticides. It should be mentioned that for a period of 5 or 6 days after treatment the persistent overnight acrid taste in the mouth could be experienced every time one worked in the crop.

It is therefore considered that under any circumstances of either commercial or experimental work where prolonged exposure to this material is likely to occur, it would be wise to take such protective measures as would reduce the opportunity for absorption by humans.

General

The experiment shows that Metasystox has considerable value as an aphicide, with little apparent danger to associated predators and parasites. (For its effect on bees, see Palmer-Jones *et al.*, 1957.) This indicates that further work under more closely controlled conditions would be justified, and it is planned to extend the work on this material accordingly.

ACKNOWLEDGEMENTS

This Division is indebted to the Bee Research Officer, Mr. T. Palmer-Jones, Wallaceville Animal Research Station, for the opportunity to participate in the observations recorded herein, and for ready co-operation extended by him and his assistants throughout.

REFERENCE

PALMER-JONES, T.; FORSTER, I. W.; GRIFFIN, L. A. M. 1957: Effect on Honey Bees of Metasystox applied from the Air as a Spray to Chou Moellier. *N.Z. J. Sci. Tech. A38:* 752-69.

DEVELOPMENT OF STREPTOMYCIN-RESISTANT VARIANTS OF *Pseudomonas syringae* van Hall IN CULTURE AND IN PEACH SEEDLINGS

By M. H. DYE, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland.

(*Received for publication, 1 August 1957*)

Summary

Streptomycin-resistant variants of *Pseudomonas syringae* emerged following serial transfer of a sensitive strain through graded concentrations of streptomycin in broth. Initially, a concentration of 0.1 to 0.5 μg./ml. streptomycin inhibited growth. After 6 transfers growth occurred at all concentrations from 0 to 679 μg./ml.

From peach seedlings previously sprayed with streptomycin and inoculated with a sensitive strain of *Ps. syringae*, 60 single colonies were isolated. Most of the isolates required more than 50 μg./ml. streptomycin to prevent growth in streak-plate assays, but only 4 of these isolates were pathogenic. One was resistant to more than 500 μg./ml., and was pathogenic.

Resistance and pathogenicity were not reduced after repeated transfer on streptomycin-free media.

Streptomycin, applied as a spray at concentrations of 100 and 500 μg./ml., did not reduce infection in peach seedlings inoculated with a strain of *Ps. syringae* resistant to 340 μg./ml. streptomycin. In the same experiment a combination of streptomycin and oxytetracycline significantly reduced infection at both 100 and 500 μg./ml. total antibiotic.

INTRODUCTION

Streptomycin-resistant bacteria often emerge during therapy of animal and human diseases with this compound. Finland (1956) published a review on the emergence of resistant strains in chronic intake of antibiotics including streptomycin. In New Zealand, horticultural formulations of streptomycin are in commercial use as prophylactic agents for a disease known as blast of stone fruit, caused by the bacterium *Pseudomonas syringae* van Hall.

This paper is concerned with the emergence of streptomycin-resistant variants of *Ps. syringae* in culture and in peach seedlings. Studies on pathogenicity, permanence of resistance, and effectiveness of combinations of streptomycin and oxytetracycline in preventing infection by resistant strains are included.

MATERIALS AND GENERAL METHODS

A highly pathogenic and streptomycin-sensitive culture of *Ps. syringae* from a single colony formed a parent strain from which all subsequent isolates and cultures were derived. Stock cultures of the parent and resistant strains were maintained on Difco nutrient agar and transferred to fresh media every 14 days. Except for studies on

N.Z. J. agric. Res. 1: 44-50.

emergence of resistant variants in liquid culture, assays for susceptibility or resistance of cultures were by the streak-plate method. In testing isolates for pathogenicity to peach seedlings, bacteria were scraped from an agar slope and smeared on 2 small areas on each of 3 leaves of a single plant. The leaves were then punctured through the bacteria with a sterilised needle. Other leaves of the same plant were inoculated with the parent organism. Cultures of unknown pathogenicity were therefore checked against a culture of proven pathogenicity to peach seedlings, on each plant. Some leaves were pricked without adding bacteria, to test the possibility of transfer of bacteria from one leaf to another. The parent strain was included on all plates when determining sensitivity of cultures to streptomycin. Streptomycin sulphate containing 710 units/mg. streptomycin was used except where otherwise stated.

EMERGENCE OF RESISTANT VARIANTS IN LIQUID CULTURE

Resistant variants were isolated *in vitro* by transferring the parent strain through graded concentrations of streptomycin in Difco nutrient broth to which was added 0.5% glucose. Inocula contained approximately 2 x 10^6 bacteria per ml. A loopful from a tube showing growth at the highest concentration in each series after 48 hours incubation at 28°c. was used to prepare inoculum for the next series. Single colonies were isolated from inoculum for the 5th transfer and tested for uniformity of resistance and pathogenicity. Results appear graphically in Fig. 1.

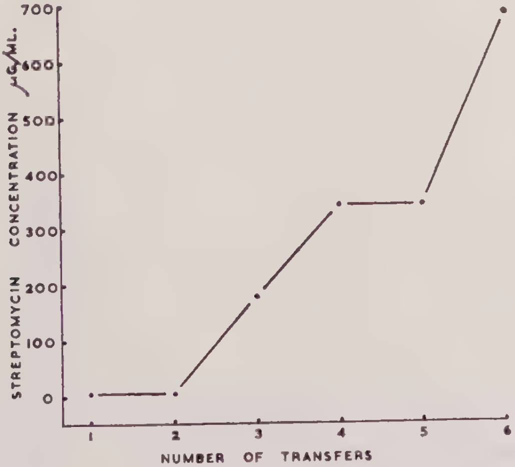


Fig. 1.—Emergence of resistant variants of *Ps. syringae* during transfer through graded concentrations of streptomycin in broth.

The minimal inhibitory concentration for the parent strain in the first assay was between 0.1 and 0.5 μg./ml. streptomycin. In the second series, using inoculum derived from a tube containing 0.1 μg./ml. streptomycin, growth occurred between 0.5 and 1.0 μg./ml. There was growth in all tubes from 0 to 175 μg./ml. in the third exposure, with inoculum

SEEDLINGS OF *Ps. syringae* ISOLATES FROM STREPTOMYCIN-TREATED PLANTS*

	Experiment Number											
	1		2		3		4		5		6	
	% *Ps. syringae* Infection											
Streptomycin Check	2 76		1 62		3 70		1 78		2 79		6 82	
	Inhibitory Concentration and Pathogenicity of Isolates											
	M.I.C. μg./ml.	P	M.I.C. μg./ml.	P	M.I.C. μg./ml.	P	M.I.C. μg./ml	P	M.I.C. μg./ml.	P	M.I.C. μg./ml.	P
Isolate No. 1	>50	—	>50	—	>50	+	300–400	—	Not determined	—	Not Determined	—
2	>50	—	>50	—	>50	—	400–500	—		—		—
3	>50	—	>50	—	>50	—	50–100	—		—		—
4	>50	—	>50	—	>50	—	30–50	—		—		
5	0.5–1.0	+	>50	—	>50	—	400–500	—		—		—
6	0.5–1.0	+	>50	—	>50	—	100–200	—		—		—
7	>50	—	>50	—	>500	+	10–30	—		—		—
8	>50	—	>50	—	>50	+	10–30	—		—		—
9	>50	—	>50	—	>50	—	400–500	—		—		—
10	>50	—	>50	—	>50	—	1–10	—		—	<1.0	+
Parent	0.5–1.0	+	0.5–1.0	+	<0.5	+	<0.5	+		+	<1.0	+

---

* In each experiment 5 seedlings were sprayed with an aqueous solution containing 50 μg./ml. streptomycin, and 5 with water. Each plant was then inoculated at 20 sites with a streptomycin-sensitive strain of *Ps. syringae*. Single-colony isolates from lesions on streptomycin-treated plants were tested for resistance to streptomycin by streak-plate assay and for pathogenicity to peach seedlings.

M.I.C. = minimal inhibitory concentration; P = pathogenicity; + = pathogenic; — = not pathogenic.

derived from a tube containing 0.5 μg./ml. streptomycin. Fifteen cultures developed from single colonies and isolated from inoculum for the fifth transfer were all resistant to 340 μg./ml. streptomycin on solid media and were all pathogenic. The bacteria grew as well on media containing 340 μg./ml. streptomycin as on media containing no streptomycin. Growth was profuse and uniform throughout the length of the streaks. After 6 transfers, growth occurred in all tubes up to 679 μg./ml.

EMERGENCE OF RESISTANT VARIANTS IN PEACH SEEDLINGS

Golden Queen peach seedlings were sprayed with an aqueous solution containing 50 μg./ml. streptomycin and inoculated with the parent organism as soon as they were dry. The method of inoculation and conditions of disease incubation were as described by Dye and Dye (1954) with the modifications of Dye (1957).

Six days after inoculation, bacteria were re-isolated from infected leaves and 10 single colonies were pricked off to agar slopes. These cultures were then tested for susceptibility to streptomycin and for pathogenicity to peach seedlings. Results of 6 experiments are recorded in Table 1.

Of a total of 60 isolates, 4 were resistant to more than 50 μg./ml. streptomycin, and were also pathogenic. The 4 cultures were all obtained in one experiment. Thirty cultures were resistant to 50 μg./ml. or more, but were not pathogenic, while 4 were inhibited by concentrations between 1 and 50 μg./ml., and were not pathogenic. Three others were pathogenic and of the same order of sensitivity as the parent culture. Nineteen cultures were not tested for resistance when they were found to be non-pathogenic. All plants were susceptible to infection by the parent organism. No pricked but uninoculated leaves became infected.

Further investigations were carried out on 1 of the 4 isolates found to be pathogenic and resistant to more than 50 μg./ml. (isolate 7, Exp. 3, Table 1). Eight cultures derived from single colonies of this isolate were all resistant to more than 500 μg./ml. streptomycin on solid media. Growth was uniform throughout the length of the streaks.

When 5 peach seedlings were sprayed with a solution of streptomycin sulphate containing 50 μg./ml. streptomycin, and each plant inoculated at 20 sites with this isolate, 85% of inoculation sites became infected. Infection in the check plants was 86%.

Since the majority of cultures from streptomycin-treated plants were not pathogenic, cultures from check plants were isolated in Exp. 5 and 6. Three out of 10 cultures from check plants were pathogenic in Exp. 5, and 6 out of 10 in Exp. 6. Thus an average of 45% of the isolates from check plants were pathogenic, while only 5% of the bacteria isolated from streptomycin-treated plants were pathogenic in the same experiments. In some experiments, 100% of isolates from check plants have been found to be pathogenic.

PERSISTENCE OF RESISTANCE AND PATHOGENICITY

Cultures which were resistant and pathogenic were retested after a number of transfers on streptomycin-free media. Results are recorded in Table 2. Resistance and pathogenicity were retained. Strains susceptible to streptomycin, including the parent organism, remained susceptible.

TABLE 2. PERSISTENCE OF RESISTANCE TO STREPTOMYCIN AND PATHOGENICITY TO PEACH SEEDLINGS BY RESISTANT VARIANTS OF *Ps. syringae* AFTER TRANSFERS ON STREPTOMYCIN-FREE MEDIA

Culture No.	Before Transfer		No. of Transfers*	After Transfer	
	M.I.C. (μg./ml.)	Pathogenicity		M.I.C. (μg./ml.)	Pathogenicity
1	>500	+	5	>600	+
2	>350	+	11	>600	+
3	679–785	+	16	>600	+
4	0.5–1	+	22	1–10	+
Parent	0.5–1	+	24	0.5–1	+

M.I.C. = minimal inhibitory concentration; + = pathogenic.
* Cultures transferred every 14 days.

EFFECTIVENESS OF COMBINATIONS OF STREPTOMYCIN AND OXYTETRACYCLINE IN PREVENTING INFECTION BY A STREPTOMYCIN-RESISTANT VARIANT

The development of resistance to single antibiotics may be delayed by combinations of 2 or more antibiotics (Jawetz 1953; Wright *et al.* 1953). English and van Halsema (1954) reported that a combination of streptomycin and terramycin (oxytetracycline) was superior to streptomycin alone in delaying emergence of resistant strains of *Erwinia amylovora* and *Xanthomonas vesicatoria* in culture.

Aqueous solutions prepared from a mixture containing 10 parts of streptomycin and 1 part of oxytetracycline were sprayed on peach seedlings at concentrations of 100 and 500 μg./ml. total antibiotic. Other plants were sprayed with solutions containing 100 or 500 μg./ml. streptomycin alone. As soon as dry, all plants were inoculated with a strain of *Ps. syringae* which had developed resistance in culture to 340 μg./ml. streptomycin. Inoculum was built up from a single colony. The experiment was performed twice with 5 plants in each treatment. Results appear in Table 3.

There was no significant difference in infection between check plants sprayed with water and plants sprayed with streptomycin at either 100 or 500 μg./ml. The combination of streptomycin and

TABLE 3. EFFECTIVENESS OF STREPTOMYCIN APPLIED ALONE AND IN COMBINATION WITH OXYTETRACYCLINE IN PREVENTING INFECTION OF PEACH SEEDLINGS BY A STREPTOMYCIN-RESISTANT VARIANT OF *Ps. syringae**

Treatment		Experiment 1 % infection †	Experiment 2 % infection †
Check		87	94
Streptomycin plus oxytetracycline‡	100 μg./ml.	27	19
	500 μg./ml.	14	0
Streptomycin alone	100 μg./ml.	86	95
	500 μg./ml.	90	86
L.S.D. 5%		16	18
1%		22	25

* Resistant by streak-plate assay to 340 μg./ml. streptomycin.

† Each figure is the number of lesions that developed out of a total of 100 inoculations on 5 plants.

‡ 10 parts streptomycin plus 1 part oxytetracycline.

oxytetracycline significantly reduced infection at both 100 and 500 μg./ml.

DISCUSSION

The finding that resistant variants emerged following exposure of *Ps. syringae* to graded concentrations of streptomycin in broth agrees with the findings of Murray *et al.* (1946) working with Gram-negative bacilli pathogenic to man. Many workers have obtained resistant variants of other bacteria using the same technique. Demerec (1948) considers that resistance in *Staphylococcus aureus* arises by gene mutation and that several genes which differ from one another in "potency" are involved. The degree of resistance depends on the "potency" of the gene concerned.

Streptomycin sulphate applied as a spray at a concentration of 50 μg./ml. streptomycin was highly effective in preventing infection by the sensitive parent strain (Table 1). Plants sprayed with 50 μg./ml. streptomycin and inoculated with a resistant variant became heavily infected. This suggests that streptomycin acts directly on the pathogen in preventing blast of stone fruit.

It was demonstrated that resistant variants were present in peach plants sprayed with streptomycin and that the majority of isolates were not pathogenic. The non-pathogenic isolates may not have been *Ps. syringae* although they had the general appearance of this organism. Nevertheless, since pathogenic bacteria were isolated more readily from check plants, further study may show a relationship between development of resistance and loss of pathogenicity in this organism.

Little difference was observed between streptomycin applied alone and streptomycin combined with oxytetracycline, in protecting peach seedlings from infection by a streptomycin-susceptible strain of *Ps. syringae* (Dye 1957), but, when streptomycin-resistant variants are present, it would appear that a combination of streptomycin and oxytetracycline is more effective than streptomycin alone.

ACKNOWLEDGEMENTS

I am grateful to Dr. H. R. Thompson for statistical analyses of results and to Miss N. A. Beaven for technical assistance.

REFERENCES

DEMEREC, M. 1948: Origin of Bacterial Resistance to Antibiotics. *J. Bact. 56:* 63-74.

DYE, D. W.; DYE, M. H. 1954: Effectiveness of Therapeutants including Antibiotics in Preventing Development of Blast of Stone Fruit (*Pseudomonas syringae* van Hall). *N.Z. J. Sci. Tech. A36:* 21-6.

DYE, M. H. 1957: Studies on Horticultural Formulations of Streptomycin. *NZ. J. Sci. Tech. A38:* 898.

ENGLISH, A. R.; VAN HALSEMA, G. 1954: A Note on the Delay in the Emergence of Resistant *Xanthomonas* and *Erwinia* Strains by the Use of Streptomycin plus Terramycin Combinations. *Plant Dis. Reptr 38 (7):* 429-31.

FINLAND, M. 1956: Emergence of Resistant Strains in Chronic Intake of Antibiotics. A Review. First International Conference on Antibiotics in Agriculture. *Nat. Acad. Sci.—Nat. Res. Coun., Wash., Publ. 397.*

JAWETZ, E. 1953: Combined Antibiotic Action: Experimental and Clinical. *Antibiotics Annual 1953–54.* pp. 41-5.

MURRAY, R.; KILHAM, L.; WILCOX, C.; FINLAND, M. 1946: Development of Streptomycin Resistance of Gram-bacilli *in vitro* and during Treatment. *Proc. Soc. exp. Biol. Med. 63:* 470-4.

WRIGHT, S. S.; PURCELL, E. M.; WILCOX, C.; BRODERICK, M. K.; FINLAND, M. 1953: Antibiotic Combinations and Resistance to Antibiotics. *J. Lab. clin. Med. 42:* 877-95.

PASTURE RESPONSES TO PHOSPHATIC FERTILISERS

By J. KARLOVSKY, Rukuhia Soil Research Station, Department of Agriculture, Hamilton.

(Received for publication, 9 October 1957)

Summary

The relative effectiveness of superphosphate and equal weights of alternative phosphate fertilisers has been determined on two Waikato soil types over a period of 4 years.

Nauru rock phosphate gave approximately half the yield increases obtained with superphosphate on Hamilton clay loam and one-third on Horotiu sandy loam. (The superphosphate responses were of the order of 16% on Hamilton clay loam and 32% on Horotiu sandy loam).

North African phosphate gave yields similar to superphosphate on unlimed ground, but was less effective on limed ground, markedly so in the case of Horotiu sandy loam.

Oxalic acid superphosphate gave inferior results on Hamilton clay loam, yielding in eight seasons significantly less than superphosphate.

Serpentine superphosphate yielded slightly but consistently less than superphosphate.

"Super compound" (75% superphosphate, 15% Nauru rock phosphate, 10% serpentine rock) gave similar results to superphosphate, except in one season on each of the soils when yields were significantly lower.

Carbonate of lime had no detectable effect on pasture production on the Nauru rock phosphate plots on either soil type.

The addition of lime to superphosphate increased pasture production on Hamilton clay loam (initial pH 5.7), but had a negligible effect on Horotiu sandy loam (initial pH 5.9).

Nauru rock phosphate greatly, and North African phosphate to a lesser extent, increased the soil phosphorus (Truog method).

On Horotiu sandy loam the soil potassium was significantly decreased by the phosphate treatments.

INTRODUCTION

When shortage of sulphur threatened the manufacture of superphosphate in 1950, the Department of Agriculture, Extension Division, commenced trials comparing finely ground Nauru rock phosphate and other phosphatic fertilisers with superphosphate.

The aim of these trials was to ascertain whether superphosphate could be successfully replaced by (a) rock phosphates, (b) mixtures of superphosphate with serpentine rock and finely ground Nauru rock phosphate, or (c) a phosphate product that does not require sulphur in its manufacture.

EXPERIMENTAL

Two trials were laid down in 1951 at Rukuhia Soil Research Station—one on each of two important Waikato soil types: Trial A on Hamilton clay loam, and Trial B on Horotiu sandy loam.

Hamilton clay loam is a semi-mature brown granular clay loam derived from volcanic ash, which is thought to be of andesitic origin. Horotiu sandy loam is an immature yellow-brown loam derived from rhyolite alluvium mixed with andesite ash (N.Z Soil Bureau, 1954).

The initial pH of the control plots on the Hamilton clay loam was 5.7 and on Horotiu sandy loam 5.9. Neither of the above soils is deficient in sulphur. Chemical analysis showed adequate S content in herbage on plots receiving no sulphur, and the addition of this element failed to increase production.

FERTILISERS

The fertilisers used and their P_2O_5 contents are given in Table 1.

TABLE 1. FERTILISERS USED AND THEIR P_2O_5 CONTENTS

Fertilisers	Total P_2O_5	Water-soluble P_2O_5	Citric-soluble P_2O_5
Superphosphate	22.4	18.4	—
Serpentine superphosphate[1]	16.7	1.8	12.2
"Super compound"[2]	22.3	8.5	14.4
Oxalic-acid superphosphate[3]	21.3	14.8	16.7
North African phosphate	26.4	—	—
Nauru phosphate	36.6	—	8.7

[1] A mixture of three parts of superphosphate and one part of serpentine.

[2] Prepared by mixing 15 parts of fresh superphosphate, 3 parts of pulverised Nauru rock phosphate, and 2 parts of pulverised serpentine. After curing, it contains 21.5% total P_2O_5 minimum, 8 to 10% water-soluble P_2O_5, and 12 to 14% citric-acid-soluble P_2O_5 (including the water-soluble fraction) (Bridger, 1951).

[3] Prepared by grinding 250 lb. finely ground Nauru rock phosphate (200 mesh) with 150 lb. of commercial oxalic acid and mixing with about 25% by weight of water in a concrete mixer. Then left in a heap for five days, dried in air, and bagged. (K. F. Hoy, pers. comm.)

History of the Fields

Trial A on Hamilton clay loam: This area was sown down to pasture in autumn 1946. Prior to the commencement of the trial, the area received 90 lb. ammonium nitrate and 3 cwt. serpentine superphosphate per acre in 1948, and 5 cwt. serpentine superphosphate per acre in 1949. The pasture was grazed by sheep from the time of sowing to the commencement of the experiment.

Trial B on Horotiu sandy loam: This area was grassed down in April 1950. It received 3 cwt. superphosphate per acre at the time of sowing and a further 3 cwt. superphosphate per acre in August 1950.

The pasture was grazed by sheep from the time of sowing to the commencement of the experiment.

Layout and Technique

Each treatment had six replications—three on unlimed and three on limed ground. The plots were arranged in a randomised block layout. The technique of mowing with return of clippings was used (Lynch, 1947). Each plot was 30 ft. by 6 ft., and out of this two mower widths of 20 in. were taken for 30 ft. of the plot length when cutting for weighing.

Soil Analysis

Soil samples were collected prior to the application of any treatment and thereafter three times on each soil type. The samples consisted of composites of 15 cores from the top three inches of each plot. The soils were analysed for pH, P, and K values.

Phosphorus was determined by the Truog method, results being reported as parts P per 50 million of the soil extract as in the Soil Advisory Service.

Sampling of Herbage for Dry-matter Determination and Botanical Analysis

After the herbage of each plot was weighed, a representative sample of approximately $\frac{1}{2}$ lb. was put into a tin with a lid to prevent moisture losses. In the laboratory a 200 g. sub-sample was taken, partly dried at 60°c. in a wind tunnel till crisp, and finally dried at 105°c. for 6 hours, after which dry matter was determined.

Herbage samples for botanical analyses were collected 13 and 15 times for trials A and B respectively. A weighed composite sample from each treatment was dissected in the green stage, dried overnight at 105°c., and the percentages calculated on the basis of the dry weight.

Treatments

1. Control
2. Superphosphate 3 cwt./year
3. North African phosphate 3 cwt./year
4. Nauru phosphate 3 cwt./year
5. Nauru 10 cwt. 1st year, thereafter 3 cwt./year
6. Serpentine superphosphate 3 cwt./year
7. "Super compound" 3 cwt./year
8. Oxalic-acid superphosphate 3 cwt./year

Half the replicates were limed, at 5 cwt./year in Trial A, and at 4 cwt./year in Trial B. Trial B also received two dressings of 2 cwt. muriate of potash (60% K_2O).

The dates of topdressings were as follows: Trial A: 7 August 1951; 6 April 1952; 8 July 1953; and 17 March 1954. Trial B: 9 May 1951;

16 May 1952; 4 July 1953; 26 March 1954. Commercial muriate of potash (60% K_2O) was applied on 22 October 1952 and 15 July 1954 at the rate of 2 cwt. per acre.

The first cut on Hamilton clay loam was made on 26 September, 1951, and the final cut on 4 January, 1956. 62 cuts were taken over this period.

The cutting of the trial on Horotiu sandy loam was commenced on 6 September 1951 and the trial was closed on 27 July 1955. A total of 51 mowings was taken over a period of just under 4 years.

TABLE 2. DRY-MATTER PRODUCTION RELATIVE TO SUPERPHOSPHATE
TRIAL A

Treatment	1	2	3	4	5	6	7	8
Spring 1951	93	100	99	94	95	96	100	97
Summer 1951-52	95	100	101	98	104	97	101	97
Autumn 1952	84*	100	92	85*	90*	94	95	91*
Winter 1952	82**	100	96	91*	91*	98	101	100
Spring 1952	95	100	99	97	102	95	96	94
Summer 1952-53	84*	100	100	95	104	92	95	93
Autumn 1953	85**	100	96	93	101	92*	92*	91**
Winter 1953	88	100	95	98	100	98	95	93
Spring 1953	91*	100	96	96	98	96	96	93*
Summer 1953-54	67**	100	95	83*	100	93	98	86
Autumn 1954	80**	100	94*	91**	94*	93*	98	87**
Winter 1954	80**	100	97	90**	96	101	99	99
Spring 1954	91**	100	100	93**	100	97	100	94*
Summer 1954-55	79**	100	93	92	98	93	101	87*
Autumn 1955	86	100	101	98	101	99	102	98
Winter 1955	76	100	92	90	93	93	92	89
Spring 1955	87*	100	96	96	100	94	98	92*
Summer 1955-56	72**	100	91	92	101	88	99	85*
Mean								
Spring	91	100	98	95	99	96	98	94
Summer	84	100	98	94	102	94	99	92
Autumn	83	100	96	92	96	95	97	91
Winter	81	100	95	92	94	97	97	96
Total 4½ year	86	100	97	94	99	95	98	93

* Diff, from superphosphate sig. at 5%.

** ,, ,, ,, ,, ,, 1%.

Treatment 1. No manure. 2. Super. 3. North African. 4. Nauru 3 cwt. 5. Nauru 10 cwt. 6. Serpentine super. 7. Super comp. 8 Oxalic super.

Total D.M. production from super (4½ year) 37,570 lb.

RESULTS

Relative Dry-matter Production

In Tables 2 and 3 the dry-matter production for various treatments is given relative to superphosphate (100) for trials A and B respectively.

TABLE 3. DRY-MATTER PRODUCTION RELATIVE TO SUPERPHOSPHATE
TRIAL B

Treatment	1	2	3	4	5	6	7	8
Spring 1951	85*	100	86*	83**	95	100	108	102
Summer 1951-52	88*	100	97	91	97	104	107	105
Autumn 1952	57**	100	80*	68**	80*	95	113	73**
Winter 1952	43**	100	72**	54**	68**	87	97	90
Spring 1952	87**	100	97	90**	93*	98	101	101
Summer 1952-53	77**	100	95	85**	89**	93*	98	97
Autumn 1953	Trial damaged by grass grub.							
Winter 1953	72**	100	97	82**	86*	98	102	101
Spring 1953	77**	100	96	85**	87**	96	101	98
Summer 1953-54	56**	100	97	73**	69**	90	108	106
Autumn 1954	57**	100	89*	75**	75**	94	100	94
Winter 1954	57**	100	92	69**	77**	95	102	102
Spring 1954	84**	100	100	88**	91*	97	102	99
Summer 1954-55	71**	100	95	80**	84**	91*	102	90*
Autumn 1955	77**	100	94	83**	85**	89**	92*	89**
Mean								
Spring	82	100	95	87	91	97	103	100
Summer	78	100	96	86	90	97	103	100
Autumn	61	100	89	76	77	93	101	89
Winter	57	100	86	68	77	93	100	97
Total 3½ year	76	100	93	83	87	96	102	98

* Diff. from superphosphate sig. at 5%.

** ,, ,, ,, ,, ,, 1%.

For treatment see Table 2.

Total D.M. production from super (3½ year) 27,100 lb.

Soil Test Results

The effects of various phosphatic fertilisers and lime on the soil tests in top three inches layer are given in Tables 4 and 5:

Botanical Analyses

Since the changes in the botanical composition of the pasture due to different treatments were of small order, the results are not presented in detail.

TABLE 4. PHOSPHATE AND POTASSIUM IN TOP THREE INCHES OF SOIL

Treatment (Mean of Lime and No Lime)	Trial A			Trial B				
	Truog P Units			Truog P Units			K	
	1953	1954	1955	1952	1954	1955	1954	1955
1. No manure	3.5	3.5	3.8	2.4	3.7	2.5	10.7	11.3
2. Super	4.6	7.5	7.0	3.7	3.7	4.0	6.7	6.3
3. N. Africa	9.5	10.7	13.3	7.6	7.8	9.0	8.2	9.0
4. Nauru 3 cwt.	19.0	20.8	26.2	7.8	14.2	14.2	9.3	8.3
5. Nauru 10 cwt.	37.7	41.8	40.2	16.3	18.2	24.2	9.5	8.7
6. Serp. super	5.1	4.7	7.0	3.5	5.2	4.7	7.3	9.2
7. Super comp.	7.4	6.7	9.5	3.6	4.2	4.2	7.3	7.8
8. Oxal. super	5.2	5.3	6.8	3.3	4.3	3.7	7.8	8.7
Sig. diff.—								
5%	4.6	6.2	3.4	1.2	5.8	1.9	1.4	2.1
1%	6.2	8.3	4.5	2.7	7.8	2.5	1.9	2.8

* Truog P units = parts P per 50,000,000 of soil extract.

TABLE 5. COMPARISON OF LIME AND NO LIME PLOTS

Trial A

	pH			Truog P		
	1953	1954	1955	1953	1954	1955
Lime	6.08		6.27	11.65	13.75	15.6
No lime	5.82		5.74	11.36	11.50	12.9
Diff.	0.26**		0.53**	0.29	2.25	2.7

Trial B

	pH			Troug P			K	
	1952	1954	1955	1952	1954	1955	1954	1955
Lime	6.18	6.07	6.09	6.0	7.6	9.0	9.1	9.1
No lime	6.04	5.77	5.78	6.1	7.7	7.7	7.6	8.2
Diff.	0.14*	0.30**	0.31**	−0.1	−0.1	1.3	1.5	0.9

* Diff sig. at 5%   ** at 1%.

DISCUSSION

Trial A on Hamilton Clay Loam

The production was measured over a period of 18 seasons (i.e. $4\frac{1}{2}$ years). However, owing to the shortage of superphosphate in 1951, serpentine superphosphate was used on treatment No. 2 in that year, so that comparisons between superphosphate and other fertilisers can be made only over a period of 16 seasons*, from autumn 1952 onwards.

Effect of Various Phosphates on Pasture Production. All fertilisers gave significant overall yield increases ranging from 8% for oxalic acid superphosphate to 16% for normal superphosphate. Superphosphate was the highest yielding fertiliser, being significantly better than control in 12 seasons.

Nauru rock phosphate at 10 cwt./acre initially was the second best treatment. It yielded significantly less than superphosphate in autumn 1952 and 1954 and in winter 1952, but in summer it was usually slightly better than superphosphate. Nauru rock phosphate at 3 cwt. yielded significantly less than superphosphate in six seasons.

North African phosphate and "Super compound" yielded significantly less than superphosphate in only one autumn season.

Serpentine superphosphate yielded consistently below superphosphate and the differences achieved significance in autumn 1953 and 1954. It should be remembered, however, that fertilisers were compared on the equal weight basis and that this fertiliser has the lowest P_2O_5 content of all fertilisers used (see Table 1).

Oxalic acid superphosphate gave very low results on this soil type, being significantly below superphosphate in 8 seasons. However, this might have been partly due to the scorching of the sward caused by this fertiliser after the topdressing in 1952 and 1954, which considerably lowered the autumn production.

Residual Effect. The last topdressing of this trial was in autumn 1954. No fertiliser was applied in autumn 1955, but the pasture production measurements were continued over the three following seasons—winter 1955, spring 1955, and summer 1955-56, in order to study the residual effect of fertilisers. Even over these seasons superphosphate has remained the highest yielding fertiliser, followed by Nauru rock phospate at 10 cwt./acre initially. The only treatment which improved its relative position was the Nauru rock phosphate at 3 cwt. initially which yielded slightly better than serpentine superphosphate.

Lime Effect. Carbonate of lime did not affect the production due to oxalic acid superphosphate, "super compound," and Nauru rock phosphate at 10 cwt./acre initially. All other treatments, superphosphate in particular, and the control, yielded more on limed ground. The overall lime increase in production was 4%. In the first year the lime response was very small, below 2%, but it increased during the course of the trial up to 9% in the last year 1955-56, when it reached significance at 5% level.

Analyses of Soil Samples. Four dressings of 3 cwt. of superphospate, serpentine superphosphate, and oxalic acid superphosphate raised the soil test for phosphorus by approximately 3 units. Super compound showed a higher increase, no doubt due to the Nauru rock phosphate which it contains. The same amount of North African phosphate increased it by 8.5 units. The application of Nauru rock phosphate raised the soil test for phosphorus to a very high level, approximately 2 units for each cwt. applied.

---

* Seasons were taken as three-monthly periods, as follows:
Winter: June, July, August. Summer: December, January, February.
Spring: September, October, November. Autumn: March, April, May.

Rock phosphates and oxalic acid superphosphate increased the pH slightly, but this was significant at 1% level. 1 ton of carbonate of lime applied on control plots over a period of four years raised the pH from 5.6 (in 1951) to 6.1 (in 1955).

Botanical Analyses of Herbage Samples. There were very small differences in herbage composition between treatments. Control plots yielded relatively less ryegrass and relatively more weeds than the treated plots, though the differences were slight. The individual analyses taken at intervals throughout the experiment showed that during the course of the trial the amount of cocksfoot (*Dactylis glomerata*) and especially browntop (*Agrostis tenuis*) increased considerably at the expense of ryegrass (*Lolium perenne*). This might be attributed to the constant mowing of the trial. There were normal seasonal changes in the herbage composition of the sward, i.e., the clover/grasses ratio was much higher in summer and autumn than in winter and spring. The effect of lime on the botanical composition of the pasture was not detectable.

Trial B on Horotiu Sandy Loam

As in the case of Trial A, serpentine super was used instead of superphosphate in 1951. The production of this trial was measured over a period of 14 seasons (i.e. 3½ years), but for the above reason the comparisons between superphosphate and other fertilisers can be made only over a period of 11 seasons, i.e. from winter 1952 onwards.

Effect of Various Phosphates on Pasture Production. In spite of the fact that this trial was conducted on a block of land very deficient in phosphate, Nauru rock phosphate gave very disappointing results. When applied on the same weight basis as superphosphate (i.e. 3cwt. per acre annually) it yielded significantly less than superphosphate in all 11 seasons (significant at 1% level). Even when applied at 10 cwt. per acre initially, it was always significantly below superphosphate.

North African phosphate was much superior to Nauru rock phosphate, especially on unlimed ground, but yielded significantly below superphosphate in 4 seasons.

Serpentine superphosphate in all seasons yielded slightly less than superphosphate (significantly less in summer 1952-3, 1954-5, and autumn 1955). This may be due to the lower per-acre applications of P_2O_5.

Oxalic-acid superphosphate yielded significantly less than superphosphate in two autumn seasons and one summer season. In autumn 1952, however, this may have been caused by burning of the sward by this fertiliser.

"Super compound" yielded significantly less than superphosphate in autumn 1955. It gave an overall yield 2% above superphosphate, but this difference is likely to be fortuitous, as neither Nauru rock phosphate nor serpentine superphosphate proved superior to superphosphate.

Effect of Lime on Production from Various Fertilisers. The application of 4 cwt. of carbonate of lime annually did not have a significant effect on the herbage production from the different treatments.

Analyses of Soil Samples. Four 3 cwt./acre annual applications of oxalic-acid superphosphate, serpentine superphosphate, "super compound", and superphosphate increased the soil test for phosphorus very slightly from the mean figure of 2.5 in control to 4.2. North African phosphate and especially Nauru rock phosphate showed a considerably greater increase, approximately 0.5 and 1 unit respectively for each cwt. of fertiliser applied. None of the fertilisers caused a significant change in pH status.

16 cwt. of carbonate of lime applied on control plots over a period of four years increased the pH from 5.8 (in 1951) to 6.1 (in 1955).

The soil-test potassium level was significantly decreased by all fertilisers. Superphosphate caused the greatest fall, significantly greater than all other treatments except Nauru rock phosphate at 3 cwt.

The lowering of the soil-test potassium level on treated plots may be due in part to the greater removal of clippings from these plots during the periods of flush growth. At that time clippings had to be discarded instead of being returned, because the bulk was too great for decomposition prior to the next cutting.

Botanical Analyses of Herbage Samples. There were very small differences in the botanical composition of the pasture due to the different treatments. Control plots showed slightly less clovers and slightly more weeds than treated plots. The detailed analyses revealed that during the course of the trial the amount of cocksfoot (*Dactylis glomerata*) increased to a great extent at the expense of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). The seasonal changes in the botanical composition of the sward were very similar to those in Trial A.

CONCLUSION

Finely ground Nauru rock phosphate proved to be inferior to superphosphate both on Hamilton clay loam and Horotiu sandy loam when applied on the same-weight basis. It gave very similar responses, about 9%, on both soil types when applied at the rate of 3 cwt./acre, in spite of the fact that Horotiu sandy loam is more deficient in phosphate and responds better to superphosphate than Hamilton clay loam. The higher initial applications of Nauru rock phosphate, as would be expected, gave an increased response. The addition of carbonate of lime did not significantly affect the production from Nauru rock phosphate.

On unlimed soils, North African phosphate gave as good responses as superphosphate, but on limed plots responses were much poorer, especially on Horotiu sandy loam.

Oxalic-acid superphosphate gave disappointing results on Hamilton clay loam, but this could be partly accounted for by burning of the sward by this fertiliser in 1952 and 1954.

On both soils serpentine superphosphate yielded slightly but consistently less than superphosphate. The differences reached significance in two seasons on Hamilton clay loam and in three seasons on Horotiu sandy loam. It should be noted that the fertilisers were applied on an equal-weight basis.

"Super compound" was very similar to superphosphate in yield being significantly lower in only one season on each soil.

Rock phosphates and oxalic-acid superphosphate significantly (1%) increased the pH of Hamilton clay loam. Rock phosphate, especially Nauru, raised the soil test for phosphorus to a very great extent, an effect noted by many other workers using the Truog extractant.

The addition of carbonate of lime gave an overall 4% increase in production on Hamilton clay loam (pH 5.7), but on Horotiu sandy loam (pH 5.9) a very slight decrease.

All phosphate treatments significantly decreased the soil test for potassium on Horotiu sandy loam.

References

Bridger, G. L. 1951: "Fertiliser Production in New Zealand". Report under sponsorship of New Zealand and the United States governments through the Smith-Mundt Act.

Lynch, P. B. 1947: *N.Z. J. Sci. Tech. A28:* 385-405.

N.Z. Soil Bureau 1954: General Survey of the Soils of North Island, New Zealand. *N.Z. Soil Bur. Bull. n.s. 5.*

THE LIGHT REGIME WITHIN PASTURES

By K. J. Mitchell and D. M. Calder*, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North.

(Received for publication, 30 August 1957)

Summary

The influence of height and form of growth of pastures on the proportion of light penetrating to various depths within them was measured with a small photocell. In order to get relatively steady light conditions and to avoid effects due to differences in angle of incidence of direct sunlight, all measurements were made on overcast days.

Where a pasture was predominantly ryegrass, with the leaves and flowering culms standing relatively erect, there was a steady decline in intensity of light with increasing depth below the top of the pasture, and much of the light penetrated to a considerable depth into the pasture. However, where there was a canopy of clover leaves, or where the tops of the grass leaves lay over to form a canopy, 85% or more of the incident light was intercepted by this canopy.

It is pointed out that the efficiency of utilisation of light by an area of pasture, as judged by quantity of tissue formed per unit of light energy, is probably highest where a large proportion of the photosynthetic tissue is in moderate shade.

Introduction

In most cases measurements of light interception by pastures have determined the proportion of daylight which penetrated through the pasture to reach the soil surface or a level close to it. Angstrom (1925) found that in a tall hay meadow 17.5% of daylight reached the base of the pasture. Weaver and Flory (1934) found that in an upland prairie 5% of the light penetrated through the vegetation. Other investigators (e.g. Allard 1947, Weaver and Clements 1938, Scheer 1953, and Brougham 1956a) have found that even less light may penetrate to the ground.

Although some evidence is deducible from measurements made by Brougham (1956a) there is little direct information available concerning the relative interception of light at various depths within different types of pasture. Such information is required for a better understanding of pasture establishment and management practices (Sears 1951), and also to assist the transposition of results from controlled climate studies to field conditions. Accordingly measurements were made during midsummer and late autumn 1956 on various pastures growing at Palmerston North.

Methods

The relative intensities of light were measured with a small (1.8 x 1.1 cm.) barrier-layer selenium photocell connected to a sensitive galvanometer. The apparatus has been described by Barrowman (1956). Measurements were taken only when there was a complete cloud cover in order that the light being radiated on to the pasture should be diffuse. Measurements were taken at five sites in each pasture. Repeated checks were made of the intensity of the total light being radiated from the sky, and if this varied by more than a few per cent, measurements were abandoned until the light became more steady. To ensure that there was very little disturbance of the grass cover, the photocell was mounted on the end of a probe three feet long. At each level of measurement this was carefully pushed into the pasture from the side and then held firmly in the correct position with a screw clamp.

---

* Present address: Botany Department, Canterbury University College, Christchurch.

Pastures are rarely uniform in density and, in many, patches of bare ground are present. In tall pastures where the long herbage generally covers such bare patches, sites for measurements were chosen at random. In the shorter pastures sites were chosen in which there was a reasonably dense cover of vegetation.

DESCRIPTION OF PASTURES EXAMINED

Accurate estimation of the height of a pasture is always difficult, for leaves and stems of grass and clover do not all grow to the same height. In this paper the height is taken as the distance above the surface of the soil at which the herbage intercepted approximately 5% of the total light.

Measurements were made in January 1956 for the following types of pasture:

(1) Short-rotation ryegrass (*Lolium perenne x L. multiflorum*) with no clover present. Height 10 in., with no flowering stems.
(2) A stand of white clover (*Trifolium repens*). Height 7½ in. A close canopy was formed at 6–7 in.
(3) Red clover (*Trifolium pratense*). Height 19 in. Canopy more open than white clover.
(4) Perennial ryegrass (*Lolium perenne*), white clover; hay pasture. Height 22 in. Clover canopy at 8 in.
(5) Pasture (4) after cutting: Growth mainly grass. Height 5 in. Bare patches were obvious.
(6) A pasture of red clover, white clover, and ryegrass. Height 18 in. Two clover canopies with the higher red-clover canopy more open than the lower white-clover one.
(7) A dense pasture of perennial ryegrass and white clover with a few broad-leaved weeds. Height 3.5 in. Bare patches present.
(8) Perennial ryegrass and white clover grazed by sheep. Height 10 in., with a few grass seed heads up to 24 in.
(9) Perennial and short-rotation ryegrass and white clover grazed by dairy cows. Height 12 in. Bare patches present.

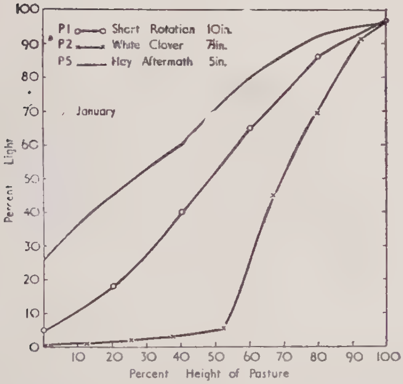
In May 1956, measurements were made on four pastures. In some cases they were adopting a form of growth not present in the equivalent pastures when examined in the summer.

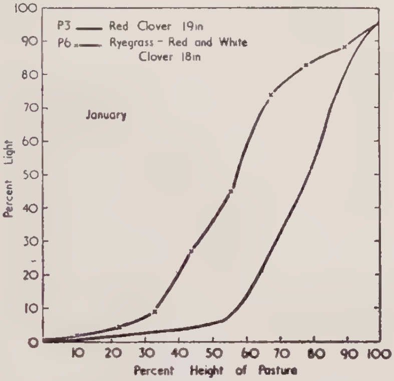
(10) Short-rotation ryegrass and white clover. Height 10 in. Leaves up to 20 in. long, but flagging caused them to lie horizontally at 9–10in.

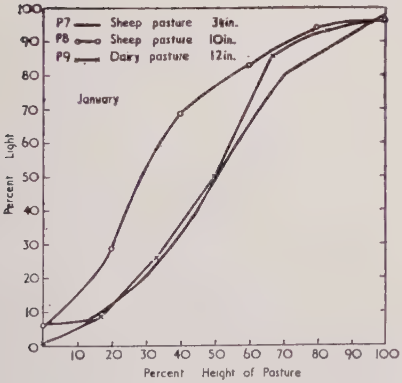
(11) Perennial ryegrass and white clover. Average height 9 in. Flagged leaves up to 14 in. long.

(12) Perennial ryegrass and white clover. Average height 9 in. No flagging.

(13) Similar pasture to 12. More recently grazed by sheep. Height 7 in. No flagging.







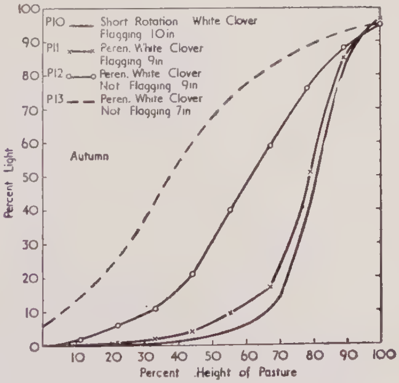


Fig. 1.—The percentage of light interception during summer within a ryegrass-dominant hay aftermath, a long pasture of short-rotation ryegrass, and a vigorous stand of white clover. In all cases the proportion of other grass or clover species present was negligible.

Fig. 2.—A comparison of the percentage of light interception in a tall pasture of ryegrass with red and white clover, and a pure stand of red clover.

Fig. 3.—Percentage of light interception in some sheep and dairy pastures during summer. All pastures ryegrass and white clover.

Fig. 4.—Influence of height of pasture, and of flagging of the tops of ryegrass leaves, on the percentage of light interception in autumn pastures.

TABLE 1. PERCENTAGES OF DAYLIGHT AT VARIOUS LEVELS IN PASTURES
Pasture numbers correspond with those in description.

Height of Measurement (in.)	Summer Pastures									Autumn Pastures			
	1	2	3	4	5	6	7†	8	9	10	11	12	13
24				99									
23													
22			100	97		100							
21													
20			99	93		98							
19													
18			90	83		95							
17													
16			64	71		88			100				
15													
14			40	63		83		100	98				
13													
12	100		18	52		69		99	96				
11										100		100	
10	97		6	42		45		96	93	98	100	99	
9										84	97	95	
8	86	100	4	34		27		94	86	46	85	88	98
7		92								14	51	76	
6	65	69	3	16	100	9		83	50	6	17	59	92
5		45			96					3	10	40	
4	40	5	2	6	92	5	100	69	26	1	4	21	75
3		3			80		90			0.5	2	11	
2	18	2	1	2	60	2	60	29	9	0.5	1	6	33
1		1			45		20			0.25	1	2	
0	5	0.5	0	1	26	1	7	6	1	0.25	0.5	1	6
Mid Past S.E.*													

* Standard errors of the measurements were nil at the top of the pasture, ran to a maximum at the centre, and if there was nearly complete light inter-
ll at the base of a pasture (n = 5).

RESULTS

The percentages of full daylight present at various heights above ground level are set out for all the pastures in Table 1. The manner in which the height, species composition, and form of growth of plants in a pasture affects the decline in relative light intensity from the top to the base of pastures is shown in Figs. 1–4. Although the angle of incidence of light does affect its penetration into a pasture (Brougham 1957), it is considered that variations in light intensity, proportion of cloud, and angle of elevation of the sun that can occur within a day or between seasons should not greatly affect the main conclusions from these results.

In a pure stand of short-rotation ryegrass in which the leaves were long and relatively erect (pasture No. 1) there was a slow initial decline in light penetration due to the isolated leaves which reached above the general body of the herbage. Once into that general body of herbage, which here reached up to 80% of "full height", the decline in light penetration became more rapid, and it was at a nearly constant rate. Five per cent of the total light reached the soil surface.

The situation is very different in a pure stand of white clover. The horizontally placed leaf blades, borne at the top of thin erect petioles, form a dense canopy which, in the pasture examined, intercepted 95% of the incident light. Below this canopy, i.e. in the bottom half of the pasture, the decline in light intensity with increasing depth is relatively small, for the petioles intercept little light.

The pattern of light interception that can occur when the above two species grow in association is shown for a hay pasture in which there was a top layer of relatively erect leaves and seed heads of grass and a bottom layer of white clover (Pasture 4). In the upper, predominantly grass layer, light penetration declined at a rate similar to that in the pasture of ryegrass alone, but once the clover layer was met the light intensity declined much more rapidly.

Cutting this hay pasture removed both the top layers of grass and the clover canopy. As the aftermath was mainly grass, it is not unexpected that the shape of its light interception curve was very similar to the top of the curve for the stand of tall ryegrass. At the time the measurements were taken, 26% of the light was penetrating through the denser parts of this aftermath, but in many other parts all the light was reaching the ground.

The differences in the form of growth of red clover and white clover were reflected in their light interception curves. Although the leaf blades of red clover are also approximately horizontal, they are spread along tall branching stems. Hence the light interception by the leaf canopy of a pure stand of red clover was not so sharp as that of white clover growing alone. Where red clover grew during summer in a long pasture in association with ryegrass and white clover, its greater height, as compared with white clover, reduced the penetration of light into the upper layers of the pasture more than occurred in the association of white clover and ryegrass only.

In the pastures examined in the autumn there was a relatively low proportion of clover present. Also the leaves of the grass plants were less rigid. Hence if a pasture became long, the tops of the grass leaves bent over and became interlaced and lay nearly horizontal at a level several inches above the base of the pasture. This formed a canopy in which light interception was almost as complete and nearly as abrupt as with white clover. In long autumn pastures of both perennial and short-rotation ryegrass, 70% of the light was intercepted by an upper zone of vegetation about two inches in depth (Fig. 4). By contrast, in pastures 12 and 13 where flagging did not occur the light penetrated much more into the pasture.

DISCUSSION

These results emphasise the large amount of shading that can occur within a pasture. Where there is a complete, or nearly complete, interception of light by the pasture, a high proportion of its photosynthetic tissue must be strongly shaded by surrounding vegetation.

In a pasture or other plant community, shading can be considered both in relation to its influence on the development of individual plants and in relation to its effect on the growth of the community as a whole.

For the great majority of crop species grown in temperate regions, anything more than a slight shading, say a 30% reduction from full daylight, almost invariably reduces the rate of growth of individual plants (e.g. Blackman and Wilson 1951). However, where the growth of such plants is considered in terms of rate per unit quantity of light energy available to the plants, it is found that the efficiency of use of light is highest at relatively low levels of light intensity. *Efficiency of use of light* is here assessed as *quantity of tissue formed per unit quantity of light energy per unit of photosynthesising leaf tissue.*

The position can be clearly illustrated by recalculation of results from the extensive series of shading trials carried out by Blackman and collaborators (e.g. Blackman and Wilson 1951). Results in conformity with the general conclusions which can be drawn from Blackman's experiments have been obtained for a number of pasture species (Mitchell 1955, 1956).

The four species for which data are presented in Fig. 5 contrast strongly in form of growth and in ecological adaptation to light. Yet in no case does maximum efficiency of utilisation of light occur at a level above approximately 30% of full daylight, and, for one species, maximum efficiency is achieved at about 15 to 20% of daylight. The experiments referred to were carried out during spring, summer, and autumn in a cool temperate climate, and the total radiation per day would have been generally within the range 250 to 350 cal/cm^2 (Black 1956). The conclusions put forward should not be extrapolated beyond that set of light and temperature conditions without further evidence. However, the situation described above is in accord with the finding, from short-duration measurements, that, for an individual leaf of plants such as those above, the maximum level of CO_2 assimilation is generally achieved at moderately low light intensities (Lundegardh 1931; Verduin and Loomis 1944).

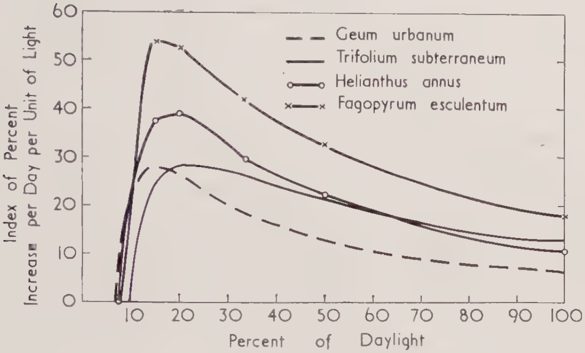


Fig. 5.—Comparison of percentage increase in dry weight per day per unit of light at various levels of shading for four species of contrasting habit of growth and ecological adaptation. Derived from results presented by Blackman and Wilson (1951).

The first requirement for the efficient use of light by the vegetation on an area of land is that all light radiated from the sky should be intercepted by photosynthetic tissue. In any higher plant community in which this occurs the total surface area of the photosynthesising tissue is far greater than that of the land on which the plants are growing. The top of the vegetation receives light at full daylight intensity but the remainder receives largely reflected and diffuse light at a much lower level of intensity. As leaves make more efficient use of light when they receive it at relatively low intensity it follows that, within limits, the larger the area or quantity of photosynthetic tissue over which the light being radiated from the sky can be spread, the greater will be the amount of plant tissue formed on a given area of land.

The higher weight of dry matter formed per unit area which is considered to occur in a moderately long pasture, as distinct from a short one, has been explained in terms of the greater length of herbage allowing complete light interception (Brougham 1956a), and of a specific defoliation effect limiting the growth of the individual tiller (Mitchell 1955). The situation illustrated in Fig. 5, and the knowledge that a similar situation holds for pasture plants, indicates that an increase in the quantity of photosynthetic tissue under moderate shading, in the range of 20 to 50% of daylight, as could occur in a longer pasture, should increase the weight of tissue formed per unit of light radiated on to the pasture.

The presence of a large quantity of photosynthetic tissue in a pasture that receives light at these levels can be achieved with a relatively deep zone of grass leaves with each leaf standing erect or nearly so. Such an objective will be opposed by the development of a shallow canopy of clover leaves over the top of the grass, or by the early stages of the formation of a canopy of blades of grass leaves over the top of a long pasture. Such a canopy intercepts most of the light, but may contain only a small proportion of the total photosynthetic tissue in a pasture, in which case most of the photosynthetic tissue is very heavily shaded.

As a pasture increases in length, increases in its efficiency of use of light would tend to be offset by an increase in the relative importance of limiting factors. Such would be the formation of a canopy of leaves, the cessation of the formation of new tillers, and the accelerated death of older leaves which is induced by shading (Mitchell 1951, Brougham 1956b).

The stage of growth of a pasture at which such limiting factors over-ride gains from an increase in area of photosynthetic tissue will depend both on species present and season of the year.

ACKNOWLEDGEMENTS

It is a pleasure to record the statistical assistance of Mr. A. C. Glenday and discussion also with Dr. P. D. Sears and Mr. R. W. Brougham.

REFERENCES

ALLARD, H. A. 1947: Light Intensity Studies in Cannan Valley, West Virginia. *Castanea 12:* 63.

ANGSTROM, A. 1925: The Albedo of Various Surfaces of Ground. *Geogr. Ann. Stockh. 7:* 323.

BARROWMAN, J. M. 1956: A Simple Portable Instrument for Measuring the Light Transmission of Pastures. *Dom. Phys. Lab.* [*N.Z.*] *Rep. R259.*

BLACK, J. N. 1956: The Distribution of Solar Radiation over the Earth's Surface. *Arch. Met., Wien. B7:* 165.

BLACKMAN, G. E.; WILSON, G. L. 1951: Physiological and Ecological Studies in the Analysis of Plant Environment. VII. An Analysis of Differential Effects of Light Intensity on the Net Assimilation Rate, Leaf Area Ratio, and Relative Growth Rate of Different Species. *Ann. Bot., Lond. n.s. 15:* 373.

BROUGHAM, R. W. 1956a: The Effect of Intensity of Defoliation on the Regrowth of Pastures. *Aust. J. agric. Res. 7:* 373.

————1956b: The Rate of Growth of Short-rotation Ryegrass Pastures in Late Autumn, Winter and Early Spring. *N.Z. J. Sci. Tech. A38:* 78.

————(In press): Interception of Light by Pure and Mixed Stands of Pasture Plants. *Aust. J. Agric. Res.*

LUNDEGARDH, H. 1931: "Environment and Plant Development." Arnold, London.

MITCHELL, K. J. 1951: *Ph.D. thesis—Univ. of Manchester, England.*

————1955: Growth of Pasture Species. II. Perennial Ryegrass, Cocksfoot and Paspalum. *N.Z. J. Sci. Tech. A37:* 8.

————1956: Growth of Pasture Species. III. White Clover, Subterranean Clover and Lotus major. *Ibid.:* 395.

SCHEER, G. 1953: Changes in Total Illumination Intensity by Foliage and Narrowing of Horizon. *Wett. u. Leben 5:* 65.

SEARS, P. D. 1951: Pasture Establishment. *Massey Agric. Coll,, N.Z. Dairy Fmg. Annu.*

VERDUIN, J.; LOOMIS, W. E. 1944: Absorption of Carbon Dioxide by Maize. *Plant Physiol. 19:* 278.

WEAVER, J. E.; CLEMENTS, F. E. 1938: "Plant Ecology." McGraw-Hill, New York.

WEAVER, J. E.; FLORY, E. L. 1934: Stability of Climax Prairie and Some Environmental Changes Resulting from Breaking. *Ecology 15:* 333.

THE EFFECT OF STILBESTROL IMPLANTS UPON THE GROWTH AND FATTENING OF ROMNEY WETHER LAMBS

By L. J. LAMBOURNE*, Ruakura Animal Research Station, Department of Agriculture, Hamilton.

(Received for publication, 23 May 1957)

Summary

The implantation of 20 mg. of stilbestrol in Romney wether lambs at docking age had only a transient effect on growth rate and no effect on liveweight at killing age. The treatment caused a slight reduction in the "finish" of lambs.

The magnitude of the response obtained was not influenced by the age and weight of lambs at time of implantation or by the use of higher dose rates of stilbestrol.

Treatment of lambs with stilbestrol is unlikely to be of any value in fat lamb production in New Zealand.

INTRODUCTION

Knowledge of the underlying endocrine regulation of many metabolic and reproductive functions of the animal has increased considerably during the last ten years. The use of hormones or of synthetic materials with a hormone-like action has been suggested as offering a revolutionary degree of control over the reproduction, lactation, growth, and fattening of animals, and with it the promise of increased efficiency and lower costs in many branches of animal production.

Much attention has been devoted to defining the mode of action and practical value of the synthetic material stilbestrol (4:4′-dihydroxy-$\alpha\beta$ diethyl stilbene) when used orally or by subcutaneous implantation in sheep, cattle, pigs, and poultry. From the evidence available, Carroll (1955)[1] in a detailed review concluded that:

> ". . . stilbestrol promotes the production of . . . a less finished carcass . . . with a higher percentage of lean tissue . . . consequent upon its . . . acceleration of protein anabolic processes . . . due to increased adrenal cortical activity . . . stilbestrol promotes the growth of early developing tissue such as viscera, bone and muscle at the expense of later-developing fat tissue, particularly during the early part of the feeding period . . ."

The implantation of 12–36 mg. has been found to increase the growth rate of lambs by up to 20% over periods of 30–90 days. The effect has been rather inconsistent in young lambs still running with the ewes, but in weaned lambs fattening on corn and roughage rations there has generally been a significant improvement in both rate of gain and efficiency of gain, with the production of a carcass of lower fat content, lower dressing percentage, and slightly less desirable conformation. In most cases, side effects have developed—notably mammary development and abnormalities of the uro-genital tract. The latter have in some cases reached serious proportions[2 3 4].

---

* Present address: C.S.I.R.O. Regional Pastoral Laboratory, Armidale, New South Wales, Australia.

N.Z. J. agric. Res. 1: 69-79.

In many parts of New Zealand the early advent of dry summer weather checks pasture growth and forces fat-lamb raisers to market lambs early at light weights. In other parts, or in other seasons, pasture growth is so well sustained that lambs, particularly the Southdown cross, can become overfat. The quick and simple injection of a stilbestrol pellet at docking time might reduce both these hazards if, under New Zealand grassland conditions, it could be relied upon to increase lamb growth rate and promote the formation of lean tissues without causing any harmful side effects.

This paper reports three trials in 1953, 1954, and 1955, with a total of about 320 Romney wether lambs from a flock of mixed-age ewes stocked at approximately six per acre on good-quality fattening pasture.

METHOD

Stilbestrol pellets were implanted subcutaneously on the neck behind the ear at docking time when the lambs were 2–4 weeks old and weighed 15–45 lb. In 1953 and 1954, 20 mg. was implanted in half the lambs and the rest were left untreated. In 1955, in an attempt to prolong or to increase the effect of the treatment, one group was given a second 20 mg. implant after 22 days, one group had an initial implant of 50 mg., while a third group was left untreated. The lambs were allotted to either of the groups in the random order in which they were picked up for docking and castration, but the number and their weight distribution were kept even. Twins were split between the groups. No attention was paid to age of dam but any lamb which, judged on its weight gain from birth to docking time, was growing poorly was excluded from the trial.

Measurements

The lambs, set-stocked with their dams on good–quality spring grass, were weighed at birth and at intervals of 2–4 weeks until they were weaned and drafted for slaughter when about 3–4 months old. At the first weighings after implantation, the lambs were checked for mammary development and for signs of rectal prolapse. At the same time, the proportion of the pellet unabsorbed was estimated by palpation.

The lambs were offered as one line, without distinguishing marks, for selection by commercial fat-lamb pickers. The carcasses were weighed and graded as for export, and in 1953 and 1954 several carcass measurements were recorded. F is the distance from the tip of the hock to the extremity of the femur, T the distance from hock to mid-crutch and G the maximum width of the gigots, at a point generally level with the tail.

RESULTS

1953 TRIAL

Growth data are shown in Fig. 1 and Table 1.

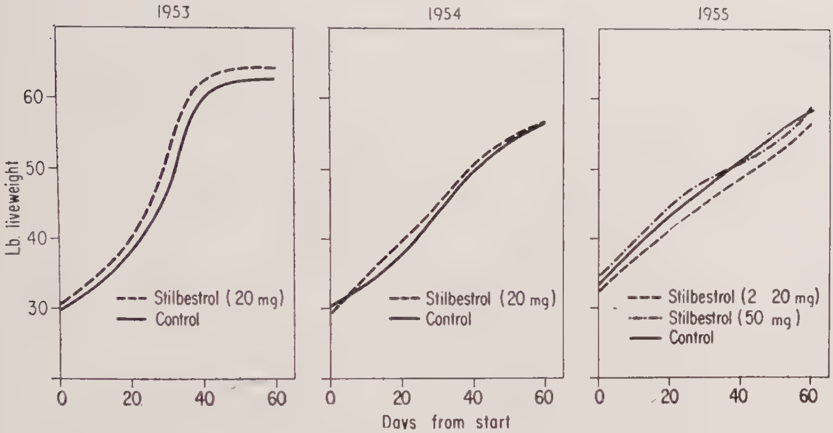


Fig. 1.—Growth curves of Romney wether lambs from docking age to the time of selection of the first slaughter draft at 4-5 months. Mean data for all lambs (singles and twins) in each group.

TABLE 1. LIVE-WEIGHT (lb.)
(Number of lambs in brackets)

	Days After Treatment			
Group	0	24	42	54
A Treated (50)	30.7	45.2	62.1	64.1
B Control (45)	30.1	42.9	60.1	62.5
Gain A		14.5*	16.9	2.0
Gain B		12.8	17.2	2.4

* Significant at 5% level.

TABLE 2. KILLING DATA 3.12.53

Group	No. Fat	Live Weight	Carcass Weight	Dressing Percentage	No. Graded		
					Down Cross	Prime	Second
Treated	13	74.0	33.4	45.1	2	11	—
Controls	23	69.8	31.1	44.6	2	16	5
Controls†	13	73.0	33.4	45.8	2	11	—

† Comparison by taking same number of lambs as were selected in the treated group.

Differences in weight gains were not significant except in the first period. All lambs are bulked in Table 1, preliminary study having shown that there was no difference in response between singles and twins.

TABLE 3. CARCASS MEASUREMENTS

Weight Range	Group	No.	Live Weight	Carcass Weight	Dressing Percentage	F (cm)	T (cm)	G (cm)
Below 30 lb.	Control	7	62.1	27.1	43.6	169	232	211
30–32.5	Treated	4	69.5	31.1	44.8	172	232	219
	Control	9	69.8	31.6	45.3	179	243	220
32.5–35.0	Treated	6	73.7	33.0	44.8	177	238	221
	Control	3	76.3	34.2	44.8	179	241	232
Over 35.0 lb.	Treated	3	80.7	37.5	46.5	184	245	231
	Control	4	78.0	35.9	46.0	183	241	227

Despite the greater mean live weight of the treated group, more lambs were picked from the control. There was no evidence of any consistent difference in carcass characteristics within weight groups, and the only notable feature of Table 3 is that the untreated group had fattened at lighter weights, 16 carcasses out of 23 being below 32.5 lb., compared with only four of the 13 from the treated group.

1954 TRIAL: See Fig. 1.

TABLE 4. LIVE-WEIGHT (lb.)
(Number of lambs in brackets)

	Days After Treatment			
Group	0	14	35	62
A Treated (65)	29.5	39.8	50.4	56.5
B Controls (65)	30.1	38.6	50.2	56.5
Gain A		10.3*	10.6	6.1
Gain B		8.5	11.6	6.3

* Significant at 5% level.

Weight gains over the first 14 days were again significantly higher in the stilbestrol-treated group, but over the following weeks this difference disappeared. Lamb growth was poor in 1954 because of adverse weather, and all the lambs were rather unfinished when drafted for killing.

TABLE 5. KILLING DATA 27.11.54

Group	No.	Live Weight	Carcass Weight	Dressing Percentage	No. Graded		
					Down Cross	Prime	Second
Treated	22	63.2	29.7	46.9	—	16	6
Control	28	61.8	29.2	47.3	—	15	13
Control†	22	63.2	30.3	47.9	—	15	7

† Comparison based on same number as were picked in the treated group.

TABLE 6. CARCASS MEASUREMENTS

Weight Range	Group	No.	Live Weight	Carcass Weight	Dressing Percentage	F (cm)	T (cm)	G (cm)
Below 30 lb	Treated	11	57.9	27.0	46.6	166	232	206
	Control	17	57.9	27.3	47.2	169	234	205
30–32.5	Treated	5	63.8	31.0	48.6	172	232	215
	Control	7	65.0	31.0	47.7	175	240	209
32.5–35.0	Treated	6	72.2	33.6	46.6	178	241	214
	Control	4	72.3	34.0	47.1	180	245	217

The second year's results were in line with those of the first year in showing that the 20 mg. stilbestrol implantation caused a transient increase of 10–20% in growth rate for 2–3 weeks followed by a slight depression in growth rate for another 3–4 weeks, so that there was very little difference at normal killing age between treated and untreated groups. The differences in numbers fat and in their carcass weights, though in the expected direction, were of neither statistical nor practical significance.

No cases of rectal prolapse or any other troubles were seen at any stage. The figures for udder development and pellet residues are shown in Table 7.

TABLE 7. UDDER DEVELOPMENT

Year	Days After Treatment	Group	Udder Development Grading				Proportion of Dose Left			
			1	2	3	4	0	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$
1953	24	Treated	25	16	6	1	22	18	7	1
		Control	37	6	—	—	—	—	—	—
1954	14	Treated	3	28	26	2	7	20	25	7
		Control	51	12	—	—	—	—	—	—

A few lambs that were released by accident without being graded make the total numbers disagree with those in Tables 1 and 4. Lambs were graded for udder growth before the operator knew to which group they belonged, and the four classes had quite clear-cut criteria:

Grade 1	No detectable mammary tissue; teats small, flaccid, and pale.
Grade 2	Perceptible pad of tissue; teats longer; some degree of "flush" but no secretion obtainable.
Grade 3	Definite enlargement of udder and teats; flushed appearance; watery secretion expressed.
Grade 4	As (3) but cloudy or milky fluid.

The figures in Table 7 justify the suggestion that the 20 mg. pellets were at least half absorbed in 14 days and about three-quarters absorbed in 24 days. The udder scores in 1953 paralleled the dose residue estimates, as would be expected in the later stages of such an absorption, but in 1954 there was a rough inverse relationship between them which suggested a more active phase of the process.

TABLE 8. RELATIONSHIP BETWEEN DOSE RESIDUES AND UDDER SCORES AT 14 DAYS (1954)

Estimated Dose Residue	0	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$
Mean udder development score	3.1 (7)	2.6 (20)	2.3 (25)	2.0 (7)

Considering all the 1953 and 1954 results, it seemed certain that the effect of a 20 mg. implant was measurable but was too temporary to make the treatment worthwhile, so in the final year attempts were

TABLE 9. LIVE-WEIGHT (lb.)
(Number of lambs in brackets)

Group	Days After Treatment				
	0	22	42	64	86
A Treated, 2 × 20 mg. (26)	32.1	41.4	48.6	56.5	57.3
B Treated, 50 mg. (28)	34.1	44.3	50.9	58.8	59.7
C Control (28)	33.4	43.1	51.0	58.6	58.9
Gain A		9.3	7.2	7.9	0.8
B		10.2	6.6	7.9	0.9
C		9.7	7.9	7.6	0.3

made to prolong the effect by repeating the 20 mg. initial implantation at the next weighing after 22 days, or to increase the effect by implanting 50 mg. initially.

1955 Trial: See Fig. 1.

None of the differences approached statistical significance in this trial, though the 50 mg. group did show an initial slight increase, followed by a depression, in rate of gain compared with the controls.

TABLE 10. Killing Data 15.12.55

Group	No. Fat	Live Weight	Carcass Weight	Dressing Percentage	No. Graded		
					Down Cross	Prime	Second
A Treated	10	68.1	30.4	44.6	—	—	10
B Treated	13	68.9	31.7	46.0	—	1	12
C Control	15	66.6	30.4	45.7	—	6	9
C Control†	10	68.5	32.2	47.0	—	6	4

† Comparison based on same number as were selected in the poorer treated group.

Stilbestrol treatment again produced a smaller proportion of fats, with lower carcass gradings, than the control group. No carcass measurements were made in this trial.

The repeated implantation of 20 mg. appeared more effective in prolonging the period of oestrogenic response, for the udder scores were appreciably higher in this group 42 days after the first treatment.

TABLE 11. Udder Gradings at Successive Weighings (1955)

Group	At 22 Days No. of Lambs Graded				At 42 Days No. of Lambs Graded			
	1	2	3	4	1	2	3	4
Treated, 2 × 20 mg.	3	13	6	2	1	7	13	3
Treated, 50 mg.	2	20	5	2	8	11	6	4
Control	24	3	—	—	25	2	—	—

In neither group was there any sign of distress or of any rectal or urinary abnormalities.

Table 12 summarises the various tables of liveweights in these experiments.

In the 1953 and 1954 trials, the rate of gain of the treated lambs exceeded that of the controls by nearly 0.1 lb. per day for the first two

TABLE 12. MEAN DAILY LIVE-WEIGHT GAINS (lb. per day)

Year	Group	Between Weighings			
		1 & 2	2 & 3	3 & 4	1 & 4
1953	Treated, 20 mg.	0.60	0.94	0.17	0.62
	Control	0.53	0.96	0.20	0.60
1954	Treated, 20 mg.	0.74	0.50	0.23	0.44
	Control	0.61	0.55	0.23	0.43
1955	Treated, 2 × 20 mg.	0.42	0.36	0.36	0.38
	Treated, 50 mg.	0.46	0.33	0.36	0.39
	Control	0.44	0.39	0.35	0.39

to three weeks but then fell below that of the controls for a further period, though this latter trend did not attain statistical significance. In 1955, the 50 mg. group again showed a brief increase, followed by a reduction, in growth rate, neither of statistical significance, while the 2 x 20 mg. group gave no response whatever. Slower-growing lambs gave no better response than faster-growing lambs in the same group; hence these results do not support the idea that response to stilbestrol is necessarily dependent upon a low initial rate of gain[5].

DISCUSSION

In all groups, both treated and control, the growth rates were poor during the latter part of the trial period, and the percentage fat enough to kill, their carcass weights, and dressing percentages were low. The latter figure was based upon the live weight straight from pasture and thus included a substantial amount of gut fill, while the lambs were Romneys, not Southdown cross. It is because the fattening of Romney lambs at these weights is not easy or automatic that they were felt to constitute a sensitive test of the oestrogen's effect. A complete evaluation would require corresponding trials with well-finished Southdown-cross lambs.

Averill[5] pointed out that significant responses to stilbestrol treatment have generally been reported from trials with slower-growing weaned lambs gaining up to 0.4 lb. per day under feed-lot conditions, while only small increases in growth rate, if any, are generally found with milk lambs gaining 0.5 0.8 lb. per day. It is not clear whether he considers that this difference in response is associated with rate of gain *per se* or that the better response of older lambs, like their slower growth rate, is due to their closer approach to endocrine maturity at puberty when the influence of oestrogens upon the pituitary (if that is in fact the mechanism of the growth stimulation) might be greater or more specific.

It was suggested from the data in Table 7 that the 20 mg. implant was at least half absorbed in 14 days and about three-quarters absorbed in 24 days, while at slaughter no trace of pellet residue was found in any lamb. It is thus likely that the lambs were metabolising approximately 0.7 mg. per day over the first two to three weeks and probably about 0.5-0.6 mg. per day over the whole absorption period, whereas the only lambs in Averill's groups that averaged over 0.5 mg. per day were the two that died from rectal prolapse. In 1955, the 50 mg. group, too, appeared to absorb the oestrogen equally rapidly, for their udder gradings (Table 11) were regressing towards those of the control group after 42 days. If only half the dose had been assimilated by then, it would imply a rate of 0.6 mg. per day for six weeks—again a potentially dangerous rate.

The stilbestrol used was marketed under the trade mark "Clinoestrol"—disc-shaped tablets, the size of an "Aspro", with an excipient. Two 10–mg. tablets were implanted for a 20–mg. dose, in an incision about half an inch long which was extended with the rounded end of a scalpel handle into a subcutaneous pouch of adequate size and which was subsequently closed with one or two stitches. A single tablet of the same size and shape containing 50 mg. stilbestrol was used in 1955. This slow and clumsy method seems to have resulted in a rapid but non-injurious assimilation, possibly because of the greater total surface area of these tablets than of the smooth cylindrical pellets supplied for injection syringes, or perhaps because of the nature of the inert excipient incorporated in the tablets. In view of the importance of a steady and not excessively high absorption rate, the form of pellet used may be critical, for the hazards of rectal or vaginal prolapse after oestrogen therapy are not to be incurred lightly. Apart from such causes, only breed differences can be invoked to account for the tolerance of Romney lambs to such high levels of oestrogen.

In earlier experiments with weaned lambs, dose rates of 12-15 mg. have given fairly consistent growth responses over periods of 60-80 days, while 6 mg. has been found to give poor results, if any.

In contrast to this, there are reports of good growth response by one- to two-year-old cattle to only 50-100 mg. of stilbestrol over periods of as much as 150 days. Grazing cattle have shown increased growth for as long as 200 days after treatment with 60 mg., with peak gains at between 30 and 80 days, while the greatest dose rate mentioned by Carroll was the 216 mg. implanted by Klosterman in young bulls during a feeding period of 192 days.

Fattening cattle thus give a greater response than weaned lambs, and the latter than milk lambs, to implanted stilbestrol, in terms of increased live-weight gains per milligramme absorbed. While this might well be due to some factor associated with species, age, or endocrine status, a simple but possibly useful explanation may be deduced from the present hypotheses of the mode of action of implanted stilbestrol.

If oestrogen does block the secretion of pituitary gonadotrophins and allows the gland to increase its output of growth hormone[5] and hence to accelerate protein anabolism[6, 7], the overall effects would be an observed decrease in the rate of deposition of fat and an increase in the rate of formation of bone, muscle, viscera, skin, and wool. The lower caloric cost of the latter than of fatty tissues would lead, even at constant caloric intake, to a greater rate and efficiency of body-tissue storage and of live-weight gain.

Rapidly growing milk lambs are already at or near their maximum feed intake, and are already depositing a relatively large proportion of their available nutrient surplus as non-fatty protein tissue, so that relatively little increase in rate of live-weight gain would be possible, however completely their fat deposition were suppressed.

Older lambs or steers under fattening conditions, on the other hand, are storing a large proportion of their surplus nutrients as fat, and there might be possible a substantial increase in rate of gain through the hormonal diversion of some of these nutrients to the calorically cheaper protein tissues, accentuated if the treatment directly or indirectly caused an increase in appetite and feed intake.

From the data of Wallace[8] and Palsson and Verges[9] on body composition, and of Clarke and McMeekan[10] and Barnicoat and Shorland[11] on carcass dissection and chemical analysis of New Zealand fat lambs, it can be shown that by a 5% reduction in deposition of fat tissue and an isocaloric formation of lean tissues an actively growing lamb which was putting on 15% of its gains as fat could increase its total tissue gains by only 5%. A fattening lamb between 60 and 70 lb. live weight is putting on rather more fat than lean tissues, and by a similar 5% reduction in deposition of fat and an isocaloric formation of lean tissues, might increase its total tissue gains by over 20%.

In this interpretation, the slightly reduced rate of gain shown by our treated lambs, following their transient initial response, might be evidence, not of a true endocrine homeostasis but simply of the rapid reconstitution of their normal fat depots as the effect of the oestrogen wore off.

It is clear that we still lack the adequate knowledge of the mechanisms of the growth response to implanted or orally administered oestrogen, which would unify and reconcile the abundant inconsistent observations in the literature.

But whatever the explanations may be, it is equally clear that treatment with stilbestrol at docking age is unlikely to be of any value in fat-lamb production on New Zealand grassland farms. Where lambs can be sold before exceeding five to six months of age, a broadly similar effect to that caused by stilbestrol treatment upon their growth rate and carcass composition can be produced, if it is desired, by the scientifically and aesthetically more elegant method of leaving ram lambs uncastrated[12].

REFERENCES

1. CARROLL, F. D. 1955: *Ceylon Vet. J. 3:* 86-96.
2. JORDAN, R. M. 1953: *J. Anim. Sci. 12:* 670.
3. BELL, T. D.; SMITH, W. H.; ERHART, A. B. (1954): *Ibid. 13:* 425.

4. CLEGG, M. T.; ALBAUGH, R.; LUCAS, J.; WEIR, W. C. 1955: *Ibid. 14:* 178.

5. AVERILL, R. L. W. 1955: *Proc. Brit. Soc. Anim. Prod. 1955*: pp. 18-29.

6. CLEGG, M. T.; COLE, H. H. 1954: *J. Anim. Sci. 13:* 108.

7. CLEGG, M. T.; CARROLL, F. D. 1956: *Ibid. 15:* 37.

8. WALLACE, L. R. 1948: *J. agric. Sci. 38:* 93, 243.

9. PALSSON, H.; VERGÉS, J. B. 1952: *Ibid. 42:* 1.

10. CLARKE, E. A.; MCMEEKAN, C. P. 1952: *N.Z. J. Sci. Tech. A33 (5):* 1.

11. BARNICOAT, C. R.; SHORLAND, F. B. 1952: *Ibid. A33 (5):* 16.

12. WALKER, D. E. K. 1953: *Ibid. A35:* 360.

NOTE ON TRANSMISSION OF APPLE MOSAIC BY NATURAL ROOT GRAFTING

By J. A. Hunter[1], E. E. Chamberlain[1], and J. D. Atkinson[2]

(Received for publication, 27 September 1957)

Little information is available concerning spread of apple mosaic in the field. However, a number of workers in different countries have reported an increase in incidence of this disease in established orchards (Bennett, 1928; Louw, 1944; Anonymous, 1954; Mulder, 1955). Blodgett (1938), working in New York State, U.S.A., recorded the number of infected trees in a group of orchards over a ten-year period and showed that there was a slow but significant increase in incidence of apple mosaic. Likewise records of infection in a Nova Scotia orchard, under normal orchard practice, showed a 50% increase over a seven-year period (Anonymous, 1948), while in England, records in three blocks of trees over a five-year period demonstrated a similar increase (Luckwill and Crowdy, 1950).

Thomas (1937), however, from observations in California, found no evidence of natural spread. Posnette and Cropley (1956) working with apple mosaic in England stated that they had not found evidence of spread from tree to tree, other than that which might be expected to occur occasionally by natural root grafting. They could see no reason to doubt that spread was usually by grafting, either naturally between roots or during propagation.

In New Zealand, where apple mosaic is widespread and incidence high, it seemed likely that there was some means of natural spread. In preliminary insect transmission studies the aphids *Myzus persicae* (Sulz), *Macrosiphum euphorbiae* (C. Thomas), and *Eriosoma lanigerum* (Hausm.) failed to bring about transmission.

As natural root grafting is known to occur with many tree species (Garner, 1950; Busgen, Munch, and Thomsen, 1929; Beddie, 1941) and has been observed with apple (Rogers and Vyvyan, 1934) it would appear that root grafting could be a means of natural spread of apple mosaic. Although we have found no reference to work giving experimental proof of transmission of apple mosaic through root grafts, other fruit tree viruses have been shown to be transmitted by this means (Hobart, 1956).

Working in England, Peren (1923) and Rogers and Vyvyan (1934) showed that root spread was normally considerably greater than branch spread and that roots from adjacent trees were frequently interlaced. Rogers (1935) found a similar root pattern in the Okanagan Valley in British Columbia where roots of 16-year-old apple trees extended over 20 ft. from the trunks. In New Zealand, root excavations made in a

---

[1] Plant Diseases Division.

[2] Fruit Research Division, Department of Scientific and Industrial Research.

N.Z. J. agric. Res, 1: 80-82.

Nelson orchard showed that roots from 20-year-old trees reached distances of up to 25 ft. from the trunks. As the normal planting distance in New Zealand is 18 or 20 ft., and vigour is such that branches of adjacent trees often meet, there must be a considerable interlacing of roots and therefore ample opportunity for root grafting to take place.

Since 1946, when investigations on apple mosaic were commenced at Mt. Albert, Auckland, many hundreds of healthy apple seedlings have been grown on an area where trees infected with apple mosaic have also been present. Except for the development of apple mosaic on five healthy trees in one trial, infection has not been observed in any of the above trees. The present note concerns this exception.

One hundred and twenty one-year-old apple seedlings were lined out in the field at ten-inch spacing in a continuous row. Eighteen months later alternate seedlings were grafted with scions carrying apple

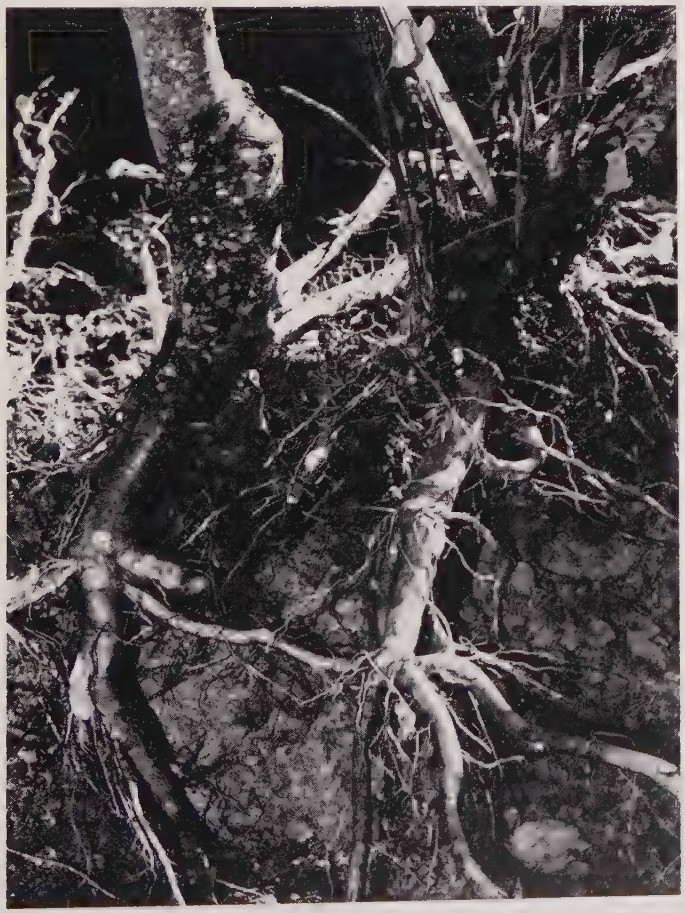


Fig. 1.—Adjacent apple trees in a nursery row showing natural root grafting which resulted in transmission of apple mosaic.

mosaic. Within four months, five ungrafted seedlings were showing typical symptoms and transmission trials confirmed that they were infected with apple mosaic. This was an obvious instance of natural transmission and was reported as such (Anonymous, 1956).

The naturally infected trees, together with those adjacent, were excavated. In all five cases the naturally infected trees were found to be connected by root grafts to adjacent diseased trees. Root union between one pair of trees is illustrated in Fig. 1. It would seem reasonable to presume that infection had been transmitted to the unworked seedlings by movement of the virus through the connecting roots. The short interval between infection of trees by top grafting and appearance of symptoms on unworked trees suggests that organic union of roots had already taken place at the time the trees were grafted.

Root grafting has been attributed by some authors to the swaying of trees in the wind, leading to rubbing and exposure of the meristematic tissue of contacting roots. This is unlikely to have been the case with these seedlings. The most probable explanation is that a root from the naturally infected tree had grown under or over one from the adjacent tree at an early stage and was later forced to unite by pressure exerted through increase in root diameter and resistance of the soil.

The relatively close spacing of trees practised in New Zealand orchards and the vigour of trees in some areas may enhance the chances of root grafting. This may be one of the factors responsible for the high incidence of apple mosaic in the Dominion.

REFERENCES

ANONYMOUS, 1948. *Rep. Sci. Serv., Dep. Agric. Can. 1947.* 105 pp. (Abstr. in *Rev. appl. Mycol. 28:* 439-40.)

———1954. *Rep. Dep. Agric., N.S.W., year ended 30th June, 1954,* 79 pp. (Abstr. in *Rev. appl. Mycol. 35:* 422.)

———1956. *Orchardist N.Z. 29: (11):* 11, 13.

BEDDIE, A. D. 1941: *Trans. roy. Soc. N.Z. 71:* 199-203.

BENNETT, C. W. 1928: *Pl. Dis. Rep., Suppl. 60:* 117-222.

BLODGETT, F. M. 1938: *Phytopathology, 28:* 937-8.

BUSGEN, M.; MUNCH, E.; THOMSON, T. 1929: "The Structure and Life of Forest Trees". 436 pp. Chapman & Hall, London.

GARNER, R. J. 1950: "The Grafter's Handbook". 224 pp. Faber & Faber, London.

HOBART, F. O. 1956: *Iowa St. Coll. J. Sci. 31:* 49-54. (Abstr. in *Rev. appl. Mycol. 36:* 197.)

LUCKWILL, L. C.; CROWDY, S. H. 1950: *Annu. Rep. Long Ashton Res. Sta. 1949*, pp. 68-79.

LOUW, A. J. 1944: *Farming in S. Africa, 19,* 32-34. (Abstr. in *Rev. appl. Mycol. 23:* 134.)

MULDER, D. 1955: *Meded. Dir. Tuinb. 18:* 446-8. (Abstr. in *Rev. appl. Mycol. 35:* 374.)

PEREN, G. S. 1923: *J. Pomol. hort. Sci. 3:* 96-102.

POSNETTE, A. F.; CROPLEY, R. 1956: *J. hort. Sci. 31:* 119-33.

ROGERS, W. S. 1935: *J. Pomol. hort. Sci. 13:* 190-201.

ROGERS, W. S.; VYVYAN, M. C. 1934: *Ibid. 12:* 110-50.

THOMAS, H. E. 1937: *Hilgardia 10:* 581-8.

THYROID FOLLICULAR CELL HEIGHT: A NOTE ON THE ABSENCE OF A SIGNIFICANT EFFECT OF *l*-THYROXINE IN ROMNEY EWES

By R. E. Munford and A. H. Kirton, Massey Agricultural College, Palmerston North

(Received for publication, 20 September 1957)

Summary

Thyroid acinar cell heights of Romney ewes were not affected significantly by restricted access to pasture, or by implants (150, 210, or 270 mg) of *l*-thyroxine.

Introduction

An investigation of the effect of restricted versus normal access to pasture, and of treatment with *l*-thyroxine upon the carcass characteristics of Romney ewes has recently been carried out at this College (Kirton, 1957). The present report notes the apparent absence of any effect of these treatments, which continued for 28 days, on the follicular cell heights in the thyroids of these sheep.

Methods

Thyroid glands were obtained at slaughter and placed in Bouin's fixative. A block of tissue was taken from one lobe and embedded in paraffin by dioxan technique. Sections (5 μ) from each thyroid were stained with haematoxylin and eosin (Cowdry, 1948). A random sampling system similar to that described by Flux *et al.* (1956) was used to select eight follicular cells for measurement in each block of tissue.

Results and Discussion

The mean thyroid cell heights for individual ewes and for ten treatment groups are shown in Table 1. Analysis of variance indicated significant differences between animals within treatments but not between treatment means. There appeared to be no obvious relationships, on a between animal basis, between mean thyroid cell height (Table 1) and thyroid gland weight or live weight changes (Kirton, 1957).

The thyroid cell heights reported here were of similar order to those observed by Butler *et al.* (1957) with Romney wethers. The between animal variance was, if anything, greater in the present instance.

Acknowledgements

The authors are indebted to Mrs. N. Cashmore for technical assistance.

The thyroxine tablets used in the investigation, of which this study forms a small part, were provided by Glaxo Laboratories, N.Z. Ltd., through the courtesy of Mr. C. B. Radcliffe.

N.Z. J. agric. Res. 1: 83-85.

TABLE 1.—EFFECT OF *l*-THYROXINE AND PLANE OF NUTRITION ON THE MEAN THYROID CELL HEIGHTS OF ROMNEY EWES

Initial Control Group[1]	Normal Access to Pasture				Restricted Access to Pasture				
	Control Group	Subcutaneous Thyroxine Implant			Control Group[2]	Subcutaneous Thyroxine Implant			Daily Injection of Thyroxine[3]
		150 mg.	210 mg.	270 mg.		150 mg.	210 mg.	270 mg.	
Means (of 8 measurements) for Individual Sheep (μ)									
5.13	3.17	4.82	5.43	4.97	5.13	4.67	4.82	5.73	4.37
7.24	6.18	4.52	4.22	5.73	5.58	3.77	3.32	4.67	5.88
6.78	6.33	3.32	3.32	4.82	5.73	4.07	4.67	4.82	5.43
6.48	4.82	5.28	6.03	6.33		6.18	3.92	5.73	3.17
6.03	5.43	5.43	4.52	5.73		6.03	4.97	4.52	
Means (± S.E.)[4] for Treatment Groups (μ)									
6.33 ± 0.41	5.18 ± 0.41	4.67 ± 0.41	4.70 ± 0.41	5.52 ± 0.41	5.48 ± 0.53	4.94 ± 0.41	4.34 ± 0.41	5.09 ± 0.41	4.71 ± 0.46

---

[1] Sheep slaughtered at the commencement of the experiment.

[2] Thyroid tissue of two animals in this group was mislaid.

[3] 5 mg subcutaneously daily; discontinued ten days before slaughter. Two sheep in this group died and the thyroid gland was not obtained from one of those.

[4] Standard errors were calculated using the estimate of variance between animals within treatments.

The investigation was in part financed by grants from the Department of Scientific and Industrial Research. One of the authors (A.H.K.) was in receipt of a N.Z. Wool Board Scholarship.

REFERENCES

BUTLER, G. W.; FLUX, D. S.; PETERSEN, G. B.; WRIGHT, E. W.; GLENDAY, A. C.; JOHNSON, J. M. 1957: Goitrogenic Effect of White Clover (*Trifolium repens* L.) II. *N.Z. J. Sci. Tech. A38:* 793.

COWDRY, E. V. 1948: "Laboratory Technique in Biology and Medicine", 2nd ed. Williams and Wilkins, Baltimore.

FLUX, D. S.; BUTLER, G. W.; JOHNSON, J. W.; GLENDAY, A. C.; PETERSEN, G. B. 1956: Goitrogenic Effect of White Clover (*Trifolium repens* L.) *N.Z. J. Sci. Tech. A38:* 88.

KIRTON, A. H. 1957: "Meat Studies in the Romney Ewe." *M. Agr. Sc. Thesis,* Massey Agricultural College.

GENETIC AND ENVIRONMENTAL COMPONENTS OF THE PRODUCTIVITY OF PERENNIAL RYEGRASS (*Lolium perenne* L.)

By S. O. FEJER, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publication, 29 August 1957)

Summary

Problems of quantitative inheritance have been studied in perennial ryegrass, with variance analysis of "autumn" and "spring" sown, spaced-planted selfings and diallel cross progenies and clonal propagules of four random plants. Except for the first cut, yields from periodical cuts were positively correlated but the low degree of significance suggested that no one cut can fully represent annual productivity. Tiller number/tiller weight relationships proved to be useful for differentiating the parents. These, and mean weight per seed and self-fertility figures are discussed from the point of view of evolution of the different types. Dangers of using clones as indicators of environmental influence in genetical trials are pointed out. Heritability estimates were generally low, but the additive genetical effects were relatively high. Genotype-environment interactions and consequent shortcomings of the heritability ratio are discussed. Reciprocal differences were of some importance in this material, but no dominance effects were observed. Changes of inbreeding depression were studied in different plants and characters; in spite of differential inbreeding influence, positive correlations were found between selfings and both overall means and maternal means of crosses.

INTRODUCTION

Since the recent revival of interest in quantitative inheritance, marked by the publication of several new methods for dealing with the problems of continuous variation, relevant studies, formerly mainly restricted to heritability of animal productivity traits (e.g. Lush 1949), have been extended to yield characters in crop plants. Some of these studies have been recently reviewed (Wallace *et al.* 1954) but the only pasture plant they quote is the annual, pearl millet (*Pennisetum glaucum*), (Burton 1951). Some information on problems in perennial grasses can be derived from breeding studies with brome grass (*Bromus inermis*), (McDonald *et al.* 1952; Hawk and Wilsie 1952) and cocksfoot (*Dactylis glomerata*), (Weiss *et al.* 1951; Kalton *et al.* 1952). It is clear that studies in quantitative inheritance have proved to be valuable for the selection and breeding procedures of the crops mentioned. No work of this kind is known with ryegrasses.

The main difficulties of quantitative inheritance studies specific to perennial pasture plants can be summarised as follows:

(a) Productivity is the regrowth after continued removal of herbage at different developmental stages and under changeable environmental conditions.

(b) This herbage offers very few clearly measurable characters which remain stable under different establishment and defoliation practices in breeding and pasture trials.

N.Z. J. agric. Res 1: 86-103.

(c) The plants are predominantly cross-fertilised and no homozygous inbred lines are available at present. Strains show an overlapping in variation.

In *Lolium*, information on genetical aspects has been published by Corkill (1950), on physiological aspects of the vegetative growth by Mitchell (1953), and on reproductive and vegetative characters by Cooper (1954).

The main purpose of the present study was to apply the methods of quantitative genetics to problems of breeding for ryegrass productivity. However, before this could be done, many of the difficulties mentioned above had to be considered.

MATERIAL AND METHODS

The parent plants were from a collection from very old pastures in New Zealand. The highly self-sterile plants (see Table 5) were mutually cross-pollinated in all possible combinations in transparent cellulose bags without emasculation. The resulting diallel-cross progenies were planted out in the field as single plants spaced 2 ft. 6 in. apart, in two adjacent layouts each of 25 randomised blocks:

(a) Three cloned parents (A, B, C) and their six reciprocal diallel-cross seedling progenies (sown in March) were planted in July 1952 ("autumn" group).

(b) Four selfed progenies of the above three and a fourth parent (D) and their 12 reciprocal diallel-cross progenies were sown in August and planted in October 1952 ("spring" group).

When six weeks old, and still in the seed boxes, the "spring" seedlings were cut and weighed. In the field, periodical cuttings and weighings of all single plants were made at intervals of 3 to 6 weeks, depending on seasonal growth rates, until April 1953. Additional data at each cutting included tiller numbers (counted in total removed herbage or in a proportionate sample), average tiller green weight, spread (calculated areas from diameter measurements of the plants after cutting), density (calculated from tiller number/area), date of emergence of the first five seed heads, number of heads in the emergence and aftermath cuts. The method of analysis of the data is an adaptation of that given by Kempthorne (1952) and is presented with the results.

RESULTS

Seasonal Green-weight Yields

The mean green weights from the several cuts of the clones and progenies are given in Table 1, and various correlation coefficients are shown in Table 2.

The plants were at different developmental stages at times of cutting; the "autumn" group continued to produce panicles during December, January, and February, at which time the "spring" plants, which had not been exposed to their cold requirements, remained in the vegetative

TABLE 1. MEANS OF GREEN WEIGHT (g) IN DIFFERENT CLONES AND PROGENIES

Plants	Sept. 1952	Nov. 1952	Dec. 1952	Jan. 1953		Feb. 1953	Apr. 1953	Total
"Autumn"								
A clone	11	197	54	Missing		88	99	449
B clone	8	164	89			95	91	447
C clone	5	51	10			12+[1]	18+[1]	96+[1] (Jan. missing)
n[2]	10	10	10			10	10	
d.05[3]	2.1	35	20					
Coeff. of var. %	27.2	37.2	40.6					
AB	18	208	73	63		74	83	519
BA	17	191	72	76		99	98	553
AC	16	214	72	81		97	102	582
CA	16	226	79	83		93	103	600
BC	19	207	82	70		74	67	519
CB	19	226	94	79		93	75	586
n	25	25	25	10		10	10	
d.05	2.7	32	8	30NS		19NS	31NS	
Coeff. of var. %	27.2	15.9	18.4					
					Coeff. of var. in Jan. 1953 %			
"Spring"								
A self	0.23			66	67.9	89	92	247
B self	0.29			97	56.9	94	91	282
C self	0.33			84	53.8	112	104	300
D self	0.14			50	69.9	75	75	200
n	32			25		25	25	
d.05	0.06			24		37NS	31NS	
AB	0.22			180	42.0	251	224	655
BA	0.28			179	48.6	226	199	604
AC	0.23			165	28.2	257	233	655
CA	0.20			182	47.0	262	246	690
AD	0.34			141	39.5	215	209	565
DA	0.28			121	33.9	192	187	500
BC	0.24			187	37.3	246	235	668
CB	0.32			218	43.9	260	226	704
BD	0.45			151	44.1	216	210	580
DB	0.42			137	48.3	185	189	511
CD	0.57			149	29.1	221	218	588
DC	0.35			134	47.6	194	202	530
n	32			25		25	25	
d.05	0.09			33		44	38	

---

[1] + for the abnormal behaviour of clone C see text. These figures have not been analysed statistically.

[2] n = number of plants used at measurement.

[3] d.05 = Difference significant at the 5% level, not to be used for progeny comparisons if marked NS (Non-significant), except for reciprocal differences where applicable.

TABLE 2. CORRELATION COEFFICIENTS BETWEEN DIFFERENT CUTS OF DIALLEL CROSSES

	"Autumn" (d.f. 4)	"Spring" (d.f. 10)
September — Total	−0.19	−0.63*
November — Total	+0.78	
December — Total	+0.61	
January — Total	+0.86*	+0.81**
February — Total	+0.55	+0.64*
April — Total	+0.21	+0.78**
September-January		−0.41
January-February		+0.81**
February-April		+0.64*
January-April		+0.78**

* Significant at the 5% level.
** Significant at the 1% level.
(*) Significant at the 10% level.

stage. Because of this difference in developmental stage, a negative correlation between the total productivities within the two groups was expected, but in fact a significantly positive correlation (r = +0.42) was obtained when calculated from the separate values of the progenies, within the five adjacent blocks of the two separate layouts. The correlations between the progeny yields at the monthly cuttings with the total yields, were all significantly positive within the "spring" group, except for the seedling cut, as were also some of the correlations of these monthly results, with the totals within both "autumn" and "spring" parts.

From Tables 1 and 2 it appears that no single cut is fully representative of total production. By contrast, Weiss *et al.* (1951) have shown, with *Dactylis glomerata,* that aftermath could be neglected and the first hay cut used. Hawk and Wilsie (1952) state that for breeding *Bromus inermis,* seedling weights were of value. With the pasture ryegrasses used in the present study, the first field cut after overwintering in the "autumn" trial was not correlated with the total production (r = −0.19); while between the early seedling cut in the "spring" trial and the total production, there was a significant negative correlation (r = −0.63*). This could be due— as suggested by Hawk and Wilsie, but not confirmed in their own case to maternal influences at the early stage of growth, among other possibilities. In the interpretation of the correlation coefficients between individual cuts and totals, the relative proportions provided at each cut must be considered.

In spite of the difficulties in choosing one cutting date as representative of total production, it was advisable to do so because tillering characters averaged over the different seasonal cuts would be very difficult to interpret. The "spring" trial was chosen for more detailed investigation because, in spring-sown plants, tillering characters are not complicated by flowering, and also there was a wider range of material investigated in this part. The January cut was finally chosen for full discussion because the cuts in January showed consistently high correlations with the total production in both parts of the experiments.

Tiller Number and Weight

Data on tiller numbers and weights are shown in Table 3.

TABLE 3. TILLER NUMBER AND WEIGHT IN DIFFERENT CLONES AND PROGENIES
(January 1953)

Plants	Tiller Number		Tiller Weight (g)	
		Coeff. of var. %		Coeff. of var. %
"Spring"				
A self	216	70.5	0.32	41.0
B self	353	58.7	0.30	36.0
C self	340	67.5	0.27	44.2
D self	293	73.1	0.28	44.9
n	25		25	
d.05	90		0.07NS	
AB	475	28.2	0.37	23.8
BA	465	30.3	0.38	29.5
AC	414	38.5	0.40	27.4
CA	444	41.3	0.42	34.3
AD	356	41.7	0.40	19.9
DA	363	37.5	0.36	31.7
BC	510	34.6	0.38	25.0
CB	566	44.2	0.40	32.1
BD	472	35.7	0.32	26.2
DB	390	40.9	0.36	30.1
CD	424	31.0	0.37	26.1
DC	428	42.6	0.31	33.6
n	25		25	
d.05	81		0.06	

For explanations see under Table 1.

There are indications from this Table that tiller number and tiller weight are not independent. Reeve and Robertson (1952) showed in their work with *Drosophila* that a thorough study of a correlation between wing and thorax length was of considerable help in partitioning and analysing environmental variability in quantitative inheritance. A similar relationship between tiller number and tiller weight, if genetically controlled, could be of particular importance in pasture plants, because they are usually grown under very close competition. Under these conditions the nutrient supply and the final size of the individual plants are strictly limited and the manner of distribution into tiller number or tiller size could strongly influence maximum pasture yield. To test this possibility in this material, correlation coefficients between tiller number and tiller weight were calculated on a progeny basis and the results are presented in Table 4.

TABLE 4. Correlation Coefficients between Tiller Number and Tiller Weight in "Spring" Plants in January 1953
(25 d.f.)

	A	B	C	D
A	−0.19	+0.52**	+0.39*	−0.32(*)
B		−0.30(*)	−0.64**	−0.40*
C			−0.30(*)	−0.02
D				+0.11

Selfings, shown in leading diagonal (23 d.f.), are not corrected for block effects as are the crosses.

There are both significant negative and positive correlations and no easy explanation seems possible. Among the negative correlations there is a highly significant one in the cross BC/CB and nearly significant ones in the selfings of both parents B and C. Positive correlations reaching significance level occur in two crosses of the parent A, but the third cross of this parent, AD/DA shows a significant negative correlation. If the data are treated graphically, as suggested by Anderson (1949) for differentiating within segregating populations, the frequency distributions of tiller number to tiller weight show an excellent differentiation between cross BC/CB and cross AD/DA. This differentiation is analogous with the usual conception of "pasture" and "hay" types, being a more precise measure of them.

The range of types occurring in this random sample affects any conclusion from this study, and merits further discussion.

The parents can be classified on the basis of a mean tillering index (tiller number/tiller weight), calculated from the January data for all possible crosses, as

B = "pasture type" with 1.30 index (high tiller number/low tiller weight)

C = "pasture-hay type" with 1.22 index (high tiller number/high tiller weight)

D = "hay-pasture type" with 1.15 index (low tiller number/low tiller weight)

A = "hay type" with 1.09 index (low tiller number/high tiller weight)

If, among the crosses studied, BC/CB and AD/DA represent stable "pasture" and "hay" types, their genetic variability should be relatively low. As green weight is the product of tiller number and tiller weight, a reduction of variability in the green weights would demonstrate more clearly any negative relationship between tiller number and tiller weight. However, the coefficients of variations for January, as shown in Tables 1 and 3, do not help to clarify the situation. On the contrary, the very high variability of the selfings suggests the possible operation of "genetic homeostasis" effects (Lerner 1954), which would tend to confound the relative genetic uniformity of the more stabilised "pasture" and "hay" types with the high resistance of the more heterozygous types to environmental changes. This factor could be operative also in the positive correlations in crosses AB/BA and AC/CA (Table 4) but the heterosis effect usually associated with it is not evident in the present material.

No final decision is in fact possible on this question of the influence of the genetic and environmental variance on the correlation between different characters without standardisation of the genotypes and environments studied.

Reproductive Data

Reproductive data will not be presented here fully, as they were obtained from the "autumn" group which did not include parent D and its progenies. The "spring" progenies remained vegetative during the trial, a few plants with D parentage being the only exceptions. However, in Table 5, seed production data of the "spring" parents are given. As number of seeds per panicle and mean weight per seed are very variable in artificial crosses, depending on conditions of pollination, and are interdependent because of competition within plants (Palmer 1952), data from the selfings only are used, where the latter distorting factor would have less effect because of the generally low percentage of fertile florets.

TABLE 5. MEANS OF SEED DATA FROM SELFINGS. "SPRING" MATERIAL.

Plants	Number of Seeds per panicle	Weight of 100 seeds (mg)
	1952	1952
A	2.5	244
B	1.4	183
C	1.7	209
D	6.1**	245

** Significantly higher (at the 1% level) than all other figures in the same column.

Parent D was significantly higher in self fertility than the other parents. The seed weights are from bulked material and have not been analysed statistically. However, two high negative correlations should be pointed out. The first was between the number of seeds/panicle (which in this case really represents self fertility) and total annual production, which approached the 5% level of significance with −0.931 for 2 d.f. The second, that between the mean weight per seed and total annual production, was lower, −0.780 for 2 d.f. Both correlations support the assumption of the clear-cut differentiation of "pasture" and "hay" types in the present material, as they can be explained as due to the evolution of these types under different conditions of tiller density, in conformity with published evidence. Gerdes (1952) found in a number of grass strains a strong relationship between "pasture type" and low mean weight per seed, while Williams (1951) discussed the causes of the correlation generally found between self sterility and perenniality in plants.

Comparison of Seedlings and Clonal Propagules

Clonal propagation is usually used for maintaining and evaluating parent material. Its advantages in quantitative inheritance trials for measuring environmental variation are obvious, analogous to the use of homozygous lines in the biometrical methods of Powers and Locke (1950), Mather (1949), and others, for representing the parents in crosses between self-fertilising plants. Clonal propagation has been used in this way by McDonald *et al.* (1952). They present heritability ratios for brome grass (*Bromus inermis*), using parent-offspring regressions, although they also report unexplained inconsistencies in other clonal tests, except for characters where vigour is not directly involved. The difficulty of using clones to represent parents in ryegrass is that the variation in plant weight resulting from the clonal division is of much the same order as that in heterozygous seedlings. In trials here it has been noted that the coefficients of variation from clonal propagules are not necessarily less than those from seedlings (Glenday, unpublished), so that estimates of heritability, based on the assumption that the variation within clones is due entirely to environmental effects, are false. Pertinent data can be found in Table 1, where the coefficients of variation for the first three cuttings in the "autumn" plants are shown separately for the three clones and for the six cross progenies. The fact that the clonal yields were more variable than those of the seedlings can be regarded as exceptional as it was obviously due to the abnormal behaviour of clone C which proved to be the best combiner in the crosses but was by far the poorest clone. It is rather unlikely that this would be caused only by segregation of productivity genes and it is thought that plant C is less tolerant to clonal propagation than the others. This is another reason why clones should not be used indiscriminately. Actually, there are indications from a subsequent selection trial that offspring of plant C are also intolerant to clonal propagation (Fejer, unpublished).

While plants vary in this respect, a certain growth inhibition after clonal propagation is of general occurrence in ryegrass and this renders difficult any direct comparison of seedlings with clones. This can be seen in Tables 1 and 3 where the totals for green weight and tiller number are consistently higher in the seedlings.

In spreading and density this interference is particularly high and these measurements will not be presented in detail. The only point of interest is the development of these characters in the "autumn" and "spring" seedlings and this is shown in the following mean figures:

These figures reflect the effect of the heading period on the "autumn" plants. Presumably some of the flower heads died after

	"Autumn" Plants		"Spring" Plants	
	Spread (cm^2)	Density (tillers/cm^2)	Spread (cm^2)	Density (tillers/cm^2)
December-January	146	1.4	102	5.1
April	214	1.8	242	3.7

cutting without being able to produce new tillers from the base for replacement, and, with their dead bases occupying the area, the density of the living tillers was reduced.

Heritability Estimates. Reciprocal Differences

The method of calculation has been mentioned in the introduction, using the following analysis of variance formulae (with p parents and k plants of each cross in each of n replications):

Source of Variation	d.f.	Expectations of Mean Squares
Blocks	(n-1)	
Parents	(p-1)	$\sigma_e^2 + k\sigma_i^2 + 2nk\sigma_s^2 + 2nk(p-2)\sigma_g^2$
Crosses within parents	½p(p-3)	$\sigma_e^2 + k\sigma_i^2 + 2nk\sigma_s^2$
Reciprocals within crosses	½p(p-1)	$\sigma_e^2 + k\sigma_i^2 + 2nk\sigma_r^2$
Block x cross interaction	(n-1)p(p-1)-1	$\sigma_e^2 + k\sigma_i^2$
Within individual crosses within blocks	np(p-1) (k-1)	σ_e^2
Total	nkp(p-1)-1	

The meanings of the variance estimates are:

σ_g^2 = variance of contribution due to a particular parent.

σ_s^2 = variance of contribution due to a particular cross.

σ_r^2 = variance of contribution to progeny due to reciprocality.

σ_i^2 = variance of contribution due to a particular cross in a particular block.

σ_e^2 — variance between different individuals of the same cross, both parents being specified.

σ_g^2 and σ_s^2 give similar information to that termed "general" and "specific combining ability" by Sprague and Tatum (1942) in corn and represent to a large degree the additive and non-additive genetical variance, σ_e^2 being a measure of mainly environmental effects. Lerner

(1950) reviewed methods used by animal breeders in calculating heritability estimates from these statistics. They sometimes did not provide for separation of the σ_s^2 term and for this reason Lush (1949) made a distinction. He called "heritability in the broad sense" the ratio of the genetic variance to the total variance in the above case. When the non-additive portion was split off, the term used was "heritability in the narrow sense". The latter ratio has been given much practical significance as expressing the fraction of the phenotypic differences between parents which may reasonably be expected to be recovered in their offspring, thus predicting the effect of mass selection. Mather (1951) recommends caution against combining heritable and non-heritable variation into such variable ratios, instead of using them as separate quantities to show what is changing the heritability of the character. Rojas and Sprague (1952) give without definition separate heritability estimates for "general" and "specific combining ability" in maize and they also stress the importance of genotype-environment interactions. Following on these lines, in Table 7, for the "spring" progenies, where σ_e^2, σ_g^2, σ_s^2 and σ_r^2 could be estimated, these are shown separately and also combined into a "heritability in the broad sense" (H_b) where

$$H_b = \frac{\sigma_g^2 + \sigma_s^2 + \sigma_r^2}{\sigma_e^2 + \sigma_g^2 + \sigma_s^2 + \sigma_r^2}$$

and into a "heritability in the narrow sense" (H_n), where

$$H_n = \frac{\sigma_g^2}{\sigma_e^2 + \sigma_g^2 + \sigma_s^2 + \sigma_r^2}.$$

To estimate genotype-environment interaction, the January tiller-weight measurements have been also analysed as 12 paired blocks with 2 plants per progeny in each and as 5 groups of 5-fold blocks with 5 plants per progeny in each. The variance estimates (with the addition of $\sigma^i{}_2 =$ variance due to interaction of genotype with environment as represented by the blocks) are also shown. The corresponding heritabilities are calculated by adding σ_i^2 to both sides of the ratio in H_b, and to the denominator only in H_n. In the "autumn" plants separation of the σ_s^2 and σ_i^2 components was not possible so that "heritability in the broad sense"

$$\frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

is given and the significance signs there refer to the differences "between crosses" in the ordinary analysis of variance only, not to a row of the special formula quoted as is the case with the "spring" plants.

Comparison of the different heritability estimates shows that with less partitioning of the variance components they tend to appear higher. This indicates the difficulty of comparing results from different investigations and, what is more important, of the shortcomings of yield prediction. While the additive genetical effects (σ_g^2) were relatively high, it is apparent that the non-additive effects (σ_s^2) were relatively unimportant in the "spring" material, except for the measurements

TABLE 6. COMPONENTS OF VARIANCE AND HERITABILITY ESTIMATES.

(a) "Spring" progenies.

Variance Estimates	Green weight			Tiller No.		Tiller weight				Spread (area)		Density	
	Jan.	Feb.	Apr.	Jan.	Apr.	Jan.	Jan. 2†	Jan. 5†	Apr.	Jan.	Apr.	Jan.	Apr.
σ_e^2	3583	5546	4680	22333	53950	103	99	109	53	648	2402	343	69
σ_g^2	547**	567**	201**	2533**	4972**	3**	3**	3**	3**	33**	430**	3**	5**
σ_s^2	- 58	−84	- 55	−266	−703	1	3	1	2(*)	138**	129	46*	6(*)
σ_i^2							5	5(*)					
σ_r^2	−2	17	−4	−50	−412	1(*)	2	1	−1	19(*)	87	9(*)	0
% H_b	12.0	8.3	2.9	9.0	6.7	5.3	11.1	8.2	7.4	23.7	21.2	14.5	13.8
% H_n	13.4	9.4	4.2	10.3	8.6	2.8	2.3	2.7	5.3	5.2	14.1	0.7	6.3

(b) "Autumn" progenies

	Green weight						Tiller Number			
	Sep.	Nov.	Dec.	Jan.	Feb.	Apr.	Sep.	Nov.	Dec.	Apr.
σ_e^2	91.36	4721	841	4109	1604	1209	1440	2769	19733	16030
σ_g^2	2.46	1204*	230**	307	44	105	134	2142**	9856*	325
% H_b	2.6	20.3	21.5	8.1	2.6	8.0	10.2	43.6	33.3	2.0
	Tiller Weight						Density			
σ_e^2	0.0079	0.090	0.124			0.0026		0.1485	0.55	0.26
σ_g^2	0.0025*	0.052**	0.009			0.0006*		0.0334(*)	0.54**	0.025
% H_b	23.7	36.7	6.7			17.9		18.4	19.4	8.6

† See text

involving spreading. This could be justification for the present strain-building procedure at Grasslands (Corkill 1957) which, with its averaging effect over a number of parental genotypes, gives weight to the additive "general combining ability" and neglects the non-additive effects which could be utilised fully only by a pedigree breeding programme. In some seasons the tillering characters showed higher heritabilities than green weight alone, a possible indication of the potential usefulness of a selection index based on several yield characters. While the density and spreading show a relatively high heritability under the spacing applied, the significance of this is doubtful because under pasture conditions, where the spreading is limited by competition, tiller density would be affected by different factors.

The relatively high heritability figures in the "autumn" material, where the non-additive effects could not be separated, might be partly due to these effects having a higher importance when the reproductive growth interacts with the vegetative, but it is more likely that the inclusion of genotype-environment interactions makes these figures appear relatively high. The probable significance of the σ_i^2 term in Table 6a points to the role of soil variation in such interactions and it is thought that seasonal variations at planting (and cutting) can cause further interactions of importance in such sensitive ratios as heritability, as pointed out in an earlier note (Fejer 1955). For that reason, in the interpretation of experimental heritability results the variability of the environments has to be taken into consideration.

As mentioned earlier, the validity of heritability estimates can be checked by selection experiments. Another method of estimating heritability, which provides for calculation of error terms, is the regression of parental clone on offspring seedlings (McDonald *et al.* 1952). This method was tried on the "autumn" plants without significant results. As mentioned previously, the variation in clonal propagation was considerable and it can be partly responsible for the high errors which made the heritability estimates non-significant.

In Table 6, the reciprocality variance components (σ_r^2) approached significance only in a few cases. However, it should be realised that they represent the overall effects of reciprocity throughout all progenies and this does not exclude significant reciprocal differences in the measurements within single crosses. Indeed, as is shown in Tables 1 and 3, such differences are rather frequent in this material. They are present, over all characters and seasons in about one-third of the cases and, if compared with the parental clones or selfings, about one-third are on the paternal side and only the remaining two-thirds can be explained by maternal influence. In some cases reciprocal differences interact with seasons to such a high degree that significant maternal influence becomes paternal and vice versa. Similar observations have been made in Petkus rye by Nürnberg-Krüger (1951) who was the first to report this in an intravarietal cross. She also supported the cytoplasmic inheritance theories of Michaelis (1951). However, as pointed out later by Michaelis (1956) himself, gamete selection could be equally responsible for such differences in heterozygous material, and specially planned experiments would be necessary to substantiate a similar claim in the present material.

Dominance and Inbreeding Effects

The use of frequency distributions in the study of quantitative characters proved to be fruitful when Boyce (1948) determined the number of major genes governing grain weight in wheat varieties, and Burton (1951) established the dominance relationships in pearl millet yield characters. Copp and Wright (1952) recommend cumulative frequency distributions on probability scale for that purpose. Smith (1952) used a simple statistical method in *Nicotiana* for estimation of "average dominance" and "potence" (see Mather 1949) in a crossing programme similar to the present one. All these methods have been tried for the January green weights, tiller numbers, and tiller weights, but the results are not presented in detail as no clear cases of dominance could be established, and the frequent skewness and bimodality of the curves presented complications which could not have been unequivocally interpreted with the heterozygous material used. It is thought that the estimations of mid-parent values were unreliable here because of differences in form and degree of inbreeding depression in the selfed progenies. However, with the low "specific combining ability" found in this material, no major dominance effects could be expected.

Differences in form of inbreeding depression are mentioned by Rebischung (1951) who states that, in ryegrass, inbreeding depression as measured by leaf width is due to elimination of the top classes

TABLE 7. Summarised Frequency Distributions in Inbred and Outcrossed Seedlings. "Spring" Plants. January 1953.

(highest classes in bold type)

	Green weight (g) class centres								
	25	75	125	175	225	275	325	375	425
Parent selfings	**41**	31	17	10	1				
Crosses	9	57	75	**78**	45	20	10	3	3

	Tiller number class centres										
	50	150	250	350	450	550	650	750	850	950	1050
Parent selfings	19	**24**	21	15	12	4	3	1	1		1
Crosses	1	22	36	65	**70**	52	31	13	2	4	2

	Tiller weight (mg) class centres								
	150	250	350	450	550	650	750	850	950
Parent selfings	23	**34**	27	9	6		1		
Crosses	10	62	**111**	76	31	7	2		1

TABLE 8. PERCENTAGE INBREEDING DEPRESSION AT DIFFERENT CUTTING DATES.

	Green Weight				Tiller No.		Tiller Weight		Spread (Area)		Density	
Plant	Sep. 52	Jan. 53	Feb. 53	Apr. 53	Jan. 53	Apr. 53	Jan. 53	Apr. 53	Jan. 53	Apr. 53	Jan. 53	Apr. 53
A	12	59	62	59	48	52	17	19	56	38	−19	28
B	15	45	59	58	26	39	20	30	10	22	18	15
C	−6	51	53	54	27	36	29	32	27	30	−8	0
D	75	64	63	63	52	48	21	34	42	46	12	8

rather than to the downward shift of the mean class with the highest frequency. The frequency distributions are presented in Table 7.

It seems obvious that the type of inbreeding depression found by Rebischung in leaf width did not occur in this material, except perhaps in the tiller weight. Neither could Rebischung's substitution of green weight by spreading in estimating the *degree* of inbreeding depression be justified in the present case (Table 8). The degree of inbreeding depression seems to vary with plant material, the particular character, and the developmental stage of the plants. Table 8, showing the depression of the selfed progeny of each plant compared with the mean of all diallel crosses, demonstrates this variation.

In Table 8, with plants A, B, and C, there was not much inbreeding depression at the early seedling stage, but the later green-weight figures indicated a depression of about 50–60%. Similar results have been reported by Jenkin (1931). Plant D is anomalous in showing the highest inbreeding depression in the early seedling stage, in spite of developing from heavy seeds. Apart from this, the inbreeding depression data give further support for grouping the parents into types represented respectively by A and D, and by B and C. In A and D, depression in tiller number is mainly responsible for the depression in green weight, while with B and C, tiller weight is depressed in a similar degree to tiller number. There is also a distinct differentiation in spreading in January, levelling out slightly in April in line with the depression shown in tiller number. Consequently tiller density, which is the ratio of these two characters, shows little sign of inbreeding depression.

It was also of interest to see how the correlations between the mean productivity of the selfed and crossed progenies of the four parents are affected by differential inbreeding effects. Correlation coefficients have been calculated on the one hand between selfings and overall parental means, and on the other hand between selfings and the maternal means

TABLE 9. Correlation between Means of Selfed and Crossed Progenies of Four Parents in January and April 1953. "Spring" Plants.
(2 d.f.)

	January			April		
	Green Weight	Tiller Number	Tiller Weight	Green Weight	Tiller Number	Tiller Weight
Between selfings and over-all parental means	+0.866	+0.997**	+0.189	+0.824	+0.914	+0.922(*)
Between selfings and maternal means	+0.954*	+0.993**	+0.501	+0.725	+0.933(*)	+0.878

of the crosses, analogous to the open-pollination test as applied by Corkill (1950). These are given in Table 9.

It seems that differential inbreeding depression did not greatly distort the additive genetic effects except in tiller weight in January. The significant correlations with the maternal means support the suitability of the "open-pollination" progeny test of Corkill, while selfing, if feasible, provides a progeny test of similar precision.

DISCUSSION

The main usefulness of heritability estimates is the information they give about the prospects of future selection for improving a character in the material used. These prospects have to be viewed against the past history of the breeding material. In the case of perennial ryegrass in New Zealand, this would cover about a century, from the early introductions and ecotype formation by natural selection, the recognition of the best ecotypes and their general use through a certification system (Levy and Davies 1929), to further improvements through plant-breeding methods (Corkill 1949). If the sample used in the present trial can be regarded as representing average unselected breeding material, the prospects of improvement of productivity in perennial ryegrass are rather low compared with that which apparently exists in other species. It is very likely that the heritability figures would be higher if a greater range of plants were used.

It is thought that the limited prospects of improvement are a consequence of the earlier natural and artificial selection which must have streamlined the strain and exhausted genetical variability to a considerable degree. While evidence from this trial shows that most of the genetical variability left is additive so that the present *breeding* method of strain building should be appropriate to utilise it without special pedigree methods, practical experience suggests that higher precision in *selection* methods might be required to achieve this aim. Whether higher precision in measuring yield itself is sufficient to accomplish this could be decided only by further work, but the present investigations suggest the usefulness of the relationship of tiller number to tiller weight. More of such costly measurements and construction of selection indices from them (Robinson *et al.* 1951) might be necessary to obtain further improvement. This question is more fully discussed, using the present and further data, in a recent paper by Glenday and Fejer (1956).

Indications of genotype-environment interactions suggest that such interactions might frustrate improvements if the selected material has to perform under different or more variable climatic, soil, and management conditions than applied in this experiment. On the other hand, for a more special purpose, heritability of yield would most likely be higher.

The many reciprocal differences, mainly in the nature of maternal influence, provide further theoretical justification for the open-pollination progeny test. The possibility of improvement through deliberate maternal selection could be also explored, in breeding programmes based on single crosses.

As there was a high correlation between progeny tests based on selfing and diallel crossing, it is suggested that where selfings can be obtained in similar material they can be used with confidence.

ACKNOWLEDGEMENTS

The writer wishes to thank Mr. A. C. Glenday, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for the modified analysis-of-variance formula, for the experimental design, and for help and discussion in all mathematical aspects of the results and their interpretation. Thanks are due to Dr. L. Corkill, Chief Plant Breeder, Grasslands Division, Department of Scientific and Industrial Research, for information on the material used and helpful discussion of the results, and to Mr. J. M. Todd for assistance with crosses and field work.

REFERENCES

ANDERSON, E. 1949: "Introgressive Hybridisation." Wiley, New York.

BOYCE, S. W. 1948: A Preliminary Study of the Inheritance of Grain Weight in Wheat. *N.Z. J. Sci. Tech. A30:* 13-22.

BURTON, G. W. 1951: Quantitative Inheritance in Pearl Millet. *Agron. J. 43:* 409-17.

COOPER, J. P. 1954: Studies on Growth and Development in *Lolium*. IV. Genetic Control of Heading Responses in Local Populations. *J. Ecol. 42:* 521-56.

COPP, L. G. L.; WRIGHT, G. M. 1952: The Inheritance of Kernel Weight in a *Triticum vulgare* Cross. *Heredity 6:* 187-99.

CORKILL, L. 1949: Pasture Improvement in New Zealand. *Emp. J. exp. Agric. 17:* 157-69.

——1950: A Comparison of Methods of Progeny Testing for Quantitative Characters in Ryegrass. *N.Z. J. Sci. Tech. A32:* 35-44.

——1957: Pasture-plant Breeding in New Zealand. *Agric. Rev., Lond. 3(6):* 31-7.

FEJER, S. O. 1955: Genotype-Environment Interactions in *Lolium perenne*. *Nature, Lond. 175:* 944-5.

GERDES, G. 1952: Absolutes Gewicht der wichtigsten Futtergrasarten. *Züchter* **22.**

GLENDAY, A. C.; FEJER, S. O. 1956: The Use of Discriminant Functions in the Selection of Pasture Plants, with Particular Reference to the *Lolium* Species. *Proc. 7th int. Grassl. Congr.*, pp. 461-70.

HAWK, W. G.; WILSIE, C. P. 1952: Parent-Progeny Relationships in Bromegrass. *Agron J. 44:* 112-8.

JENKIN, T. J. 1931: The Method and Technique of Selection, Breeding, and Strain-building in Grasses. *Imp. Bur. Pl. Gen., Herb. Pl. Bull. 3:* 5-34.

KALTON, R. R.; SMIT, A. G.; LEFFEL, R. G. 1952: Progeny Relationship of Orchard-grass Clones. *Agron. J. 44:* 481-6.

KEMPTHORNE, O. 1952: "The Design and Analysis of Experiments." Wiley, New York.

LERNER, I. M. 1950: "Population Genetics and Animal Improvement." C.U.P.

——1954: "Genetic Homeostasis." Oliver and Boyd, Edinburgh. London.

LEVY, E. B.; DAVIES, W. 1929: Strain Investigations Relative to Grasses and Clovers. *N.Z. J. Agric. 39:* 1-8.

LUSH, J. L. 1949: Heritability of Quantitative Characters in Farm Animals. *Proc. 8th int. Congr. Gen., Hereditas Suppl.*, pp. 356-75.

MATHER, K. 1949: "Biometrical Genetics." London.

————1951: Review of "Population Genetics and Animal Improvement", by I. M. Lerner, *Heredity 5:* 293-6.

McDONALD, E. D.; KALTON, R. R.; WEISS, M. G. 1952: Inter-relationships of Selected Bromegrass Clones. *Agron. J. 44:* 20-5.

MICHAELIS, P. 1951: Plasmavererbung und Heterosis. *Z. Pflanzenz. 30:* 250-274.

————1956: "Plasmatische Vererbung" in "Handbuch der Pflanzenzüchtung," p. 141 P. Parey, Berlin.

MITCHELL, K. J. 1953: Influence of Light and Temperature on the Growth of Ryegrass. I. Pattern of Vegetative Development. *Physiol. Plant. 6:* 21-46.

NURNBERG-KRUGER, U. 1951: Ueber die Auswirkung des Plasmas auf Leistungsmerkmale beim Roggen. *Züchter 21:* 232-40.

PALMER, T. P. 1952: Population and Selection Studies in a *Triticum* Cross. *Heredity 6:* 171-85.

POWERS, L.; LOCKE, L. F. 1950: Partitioning Method of Genetic Analysis Applied to Quantitative Characters of Tomato Crosses. *U.S. Dep. Agric. Tech. Bull. 998:* 1-56.

REBISCHUNG, J. 1951: Etude de Population de ray-grass. *Ann. Inst. nat. Rech. agron., Paris, 1:* 491-547.

REEVE, E. C. R.; ROBERTSON, F. W. 1952: Analysis of Environmental Variability in Quantitative Inheritance. *Nature, Lond. 171:* 874.

ROBINSON, H. F.; COMSTOCK, R. E.; HARVEY, P. H. 1951: Genotypic and Phenotypic Correlations in Corn and their Implications in Selection. *Agron. J. 43:* 282-7.

ROJAS, B. A.; SPRAGUE, G. F. 1952: A Comparison in Variance Components in Corn Yield Trials. III. General and Specific Combining Ability and their Interaction with Locations and Years. *Agron. J. 44:* 462-7.

SMITH, H. H. 1952: Fixing Transgressive Vigour in *Nicotiana rustica. In* "Heterosis", GOWEN, J. W. (Ed.). Iowa State Coll. Press, Ames, Iowa.

SPRAGUE, G. F.; TATUM, L. 1942: Combining Ability in Corn. *J. Amer. Soc. Agron. 34:* 923-32.

WALLACE, A. T.; MIDDLETON, G. K.; COMSTOCK, R. E.; ROBINSON, H. F. 1954: Genotypic Variances and Covariances of Six Quantitative Characters in Oats. *Agron. J. 46:* 484-8.

WEISS, M. G.; TAYLOR, L. H.; JOHNSON, I. J. 1951: Correlation of Breeding Behaviour with Clonal Performance of Orchard-grass Plants. *Agron. J. 43:* 594-602.

WILLIAMS, W. 1951: Genetics of Incompatibility in Alsike Clover, *Trifolium hybridum. Heredity 5:* 51-73.

GENETIC VARIATION AND COVARIATION IN PRODUCTIVE CHARACTERS OF NEW ZEALAND ROMNEY MARSH SHEEP

By A. L. RAE, Massey Agricultural College, Palmerston North.

(Received for publication, 20 September 1957)

Summary

Estimates of heritability of six characteristics and the genetic and phenotypic correlations among them have been obtained from a flock of New Zealand Romney Marsh sheep. The characters studied were greasy-fleece weight, staple length, quality number, fleece quality, hairiness, and body type. The characters were recorded at 14 months of age on 697 ewe hoggets which had been shorn as lambs.

Fleece quality was the only character in which there was a significant interaction between sires and years. The cause of this interaction could not be definitely decided from the data.

Only records taken at mature ages were available for some of the dams. Hence heritabilities calculated from the regression of hogget record of daughter on mature record of dam could be compared with those calculated from the regression of hogget record of daughter on hogget record of dam. Though heritabilities from the latter method were generally higher, the differences were not significant. The variance of mature records was significantly greater than that of hogget records for fleece weight only. The combined estimates of heritability were: quality number, 0.27; staple length, 0.35; fleece quality, 0.22; hairiness, 0.63; body type, 0.14. For fleece weight, the heritability from regression on mature record of dam was 0.11 while, from the regression on hogget record of dam, it was 0.31.

The genetic correlations which are expected to limit improvement from selection result in the following associations: selecting for finer wool results in shorter staple length and lower fleece weight; selecting for higher fleece weight, longer staple, or coarser wool, leads to increased hairiness.

INTRODUCTION

Despite the importance of the Romney Marsh breed in the sheep industry of New Zealand, few attempts have been made to assemble the facts needed to define the problems involved in breeding for the improvement of productivity of the breed. Stevens (1948) has reviewed in detail the organisation of the ram-breeding flocks and has stressed the importance of a relatively small number of flocks which act as a "nucleus" in supplying a high proportion of sires used in other registered flocks. Goot (1946, 1952) has published a series of studies presenting data on reproductive rates, age compositions, and generation intervals for a number of flocks. McMahon (1943, 1946) and Rae (1948) have studied the heritability of some fleece and body characters while Wright and Stevens (1953) have studied lifetime records of fleece weight and breeding performance.

The above studies have served both to outline the general structure of the breeding industry and to indicate where information of a more specific nature is lacking. In particular, any investigation of the efficiency of selection requires information on the extent of the genetic variation and covariation among the productive characters of the breed.

N.Z. J. agric. Res. 1: 104-123.

Heritability in the narrow sense is defined by Lush (1941, 1949) as the fraction of the observed phenotypic variance of a character which is caused by additively genetic differences between individuals. It is thus a measure of the additively genetic variance within the population and can be used to predict the rate of genetic gain resulting from selecting for the character. For these predictions to be reasonably accurate, it is necessary that selection be for this character alone or that the character be uncorrelated genetically with any of the other characters affecting productivity or fitness of the animal. In the latter case, the change resulting from selection for one character may be altered by selection for a genetically correlated character, thus making the actual genetic gain different from the expected genetic gain as computed from knowledge of the heritability alone. Since selection for one character alone is a condition which is seldom fulfilled in any livestock-breeding enterprise, it is obvious that heritabilities are not sufficient to describe adequately the genetic properties of a population. A more adequate description must include also the genetic covariances or correlations between the traits considered in selection. Statistically, the characters may be regarded as having some multivariate distribution of additively genetic values which requires for its specification a matrix of genetic variances and covariances. Because genetic correlations are in standardised form and are thus more easily interpreted and compared, they are used in preference to genetic covariances.

The literature on the heritability of productive traits in sheep has been reviewed by Serra (1948), Morley (1951), and Rae (1956). Studies on the Romney Marsh breed under New Zealand conditions have been reported by McMahon (1943) and Rae (1948), while Rasmussen (1942) investigated heritability of fleece weight in range Romney crossbreds in Alberta, Canada.

SOURCE OF DATA

The data used in this study were obtained from a flock of Romney Marsh sheep started at Massey Agricultural College, Palmerston North, in 1944 to investigate methods of breed improvement. The period covered in this present investigation is from 1944 to 1948.

In 1944, 250 five-year-old ewes and 150 two-tooth ewes were purchased to found the flock. In 1945, the ewe flock for this year was completely replaced by the purchase of 450 more five-year-old ewes. In 1946, 160 of these ewes from the previous year were culled and replaced by two-tooth ewes born in the flock in 1944. All older ewes were culled in the 1947 season and the flock then consisted entirely of two-year-old and three-year-old ewes born in the flock in 1944 and 1945.

The 19 sires used in the flock during the period under review were chosen from nine stud Romney flocks from the Manawatu-Wairarapa area which is the location of about 60% of the Romney Marsh pedigree flocks in the North Island of New Zealand (Stevens 1948). In the first year, ten sires were mated to 40 ewes each, the ewes being allotted at random to each sire. Seven of these sires were used in the 1945 mating season and six of them in the 1946 season. In 1945, two new sires were brought in, one of which was used only in this year, while the other was in use in both 1945 and 1946. In 1946, a further new sire was brought into service. In 1947, six two-tooth rams were used in accordance with a modification of the experimental plan. In all years, the ewes were randomised to each sire group.

The mating season started between 15 and 25 March in each year, and the rams were taken out after eight weeks. The flock was grazed throughout the winter on pasture with no supplementary feeding. The lambs were born in August and September and remained with their dams till weaning in January. At this time, weaning records on quality number, staple length, and fleece quality were taken by a committee of two or three experienced judges. The lambs were then shorn and fleece weights recorded. Within two weeks after shearing, the lambs were scored for body type.

About mid October, quality number, staple length, and fleece quality were recorded prior to shearing. At this time, a staple of wool was clipped from the region of the britch for determining hairiness. Greasy-fleece weights were taken at shearing and body type was scored a few days later. The ewe flock was shorn approximately one month later, the same routine of recording data being followed.

In general, the system of management was typical of that under which a flock is handled in the Manawatu area, except that no effort was made to allot the lambs that were doing poorly at weaning, or later, to a separate group to give them special attention and feeding. Every attempt was made to treat all lambs and ewes alike as far as the routine practices of management such as drenching against parasites, dipping, and care of feet were concerned.

Greasy-fleece weight, quality number, staple length, fleece quality, hairiness, and body type have been analysed in this study. Since all animals were shorn at weaning, the measurements of fleece production represent only the growth occurring in the ten months between weaning and hogget shearing in October of each year.

1. Greasy-fleece weight was measured to the nearest tenth of a pound immediately after the fleece was removed from the sheep. The belly wool was included in this weight. No attempt was made to obtain clean-fleece weight, since earlier studies had shown that the correlation between greasy-fleece weight and clean-fleece weight under conditions where the fleece was free from seed and burrs was high (0.93 according to Barton, pers. comm.).

2. Staple length was measured with a rule to the nearest half-centimetre on the mid-side region of the fleece prior to shearing. Since the tip of the staple in Romney wool tapers toward a point, the measurement was made from the skin to a position midway between the point where the staple starts to taper and the tip, taking care not to stretch the staple unduly.

3. Quality number, or count. For the present study, quality number was defined as the visual impression of fibre fineness judged by spreading

the fibres of individual staples on the mid-side region of the fleece. The standards generally in use in the wool trade in New Zealand were used.

4. Fleece quality. This character consisted of a visual impression of the evenness, size and type of crimping of the staples, lustre, handle, and freedom from tippiness as judged by opening the fleece at the shoulder, side, and hindquarter positions. A range of 14 grades was used. The estimate of fleece quality gave an evaluation of a number of features which are considered to be significant in manufacturing and are included in the wool buyer's concept of "style" (Henderson and McMahon 1947).

A study of the repeatability of the fleece-quality grading showed that the intra-class correlation between repeated observations by the same observers was 0.64 (Clarke *et al.* 1953). The accuracy of the observation is not high but it was used since it measured a trait of real economic importance in fleece production for which no objective measurement has yet been developed.

5. Hairiness. An objective measure of the amount of hair present in the fleece was obtained by use of the medullameter (McMahon 1937). Throughout the present investigation, samples from Position No. 5 on the hindquarter have been used. Goot (1945a) has shown that hairiness measured at this position is correlated to the extent of 0.9245 with total fleece hairiness. Previous studies have shown that the mean and variance of groups of sheep for the measurement of hairiness derived from the medullameter are strongly correlated. A logarithmic transformation has been found to make the mean and variance independent and has been used in all analyses of hairiness in this work.

6. Body type. The body-type grading referred to the degree to which the body shape of the animal approached accepted standards of desirable meat conformation. The highest development of body type required the animal to be short in length of leg with well-filled hindquarters, and flat and broad across the loin, back, and shoulders. A range of 14 grades was used in describing this character. A repeatability study showed that the intra-class correlation between repeat gradings on the same animal was 0.81 (Rae 1946). A correlation of 0.70 was also found between the body-type score and carcass grade as determined on the same group of animals after slaughter (Rae 1946). The carcass grading referred to is that described by Barton (1947).

METHOD OF ANALYSIS

Complete records were available for quality number, staple length, greasy-fleece weight, fleece quality, hairiness, and body type for 697 ewe hoggets, the records having been collected in the years 1945 to 1948 inclusive. The hoggets were the offspring of 19 sires used in the flock during the period under investigation.

As a preliminary step in the analysis, it was considered necessary to test the homogeneity of variances for each character from year to year, using Bartlett's test as described by Snedecor (1946). Body type was the only trait in which the variances were not homogeneous, the discrepancy being due to smaller variance in the year 1948. The reason for this difference is not clear. Since there appeared to be no simple transformation which could be used to produce homogeneity of variance, it was decided to analyse the data for the 1948 season separately from the remaining years.

Lush (1949) has described the various methods which are available for estimating heritability. In this investigation, two methods were used: Doubling the regression of offspring on dam and multiplying the paternal half-sib correlation by four. In estimating the genetic correlations between the characters, the relationships of paternal half-sib and offspring dam were also used. Hence, subsequent analysis of the data was carried out in two stages. Firstly, the variances and covariances between sires were analysed in order to find the heritability of each trait and the genetic correlation between each pair of traits. Secondly, the regressions of daughter on dam were computed to determine heritabilities and genetic correlations.

Estimation of Heritabilities and Genetic Correlations by the Paternal Half-Sib Method

The observations on the hoggets could be classified according to the following factors: year in which the observation was made, sire of the animal, type of birth (whether single, twin reared as a single, twin or triplet reared as a twin) and age of dam (whether five-year-old or two-year-old). In addition, age of the hogget at shearing was included as an independent variable. Since some of the sires were used in more than one year, it was considered desirable to investigate and make allowance for any interaction between sires and years.

There was a marked disproportion in the number of animals included in each of the above subclasses. In order to take this disproportion in the subclass numbers into account, the method of fitting constants by least squares as described by Hazel (1946) and Kempthorne (1952) was used.

Since the techniques used in the later analysis depended on whether the interaction between sires and years could be assumed zero, the test of significance of this interaction was made first.

Where the sire-year interaction was found to be non-significant the sire effects are considered to be randomly drawn items from a population having zero mean and a variance σ_s^2 which is to be estimated from the data. In a population mating at random, $4\sigma_s^2$ estimates the genic variance of the population. Heritability is therefore calculated as the ratio

$$\frac{4\sigma_s^2}{\sigma_s^2 + \sigma_e^2}$$

Since the effects due to years, type of birth, age of dam and age at shearing are regarded as fixed effects, the variance component for sires (σ_s^2) was estimated from the sum of squares between sires using

Method 3 as given by Henderson (1953). The error variance (σ_e^2) was estimated from the error sum of squares.

When the sire-year interaction was found to be significant, the sum of squares between sires contains an additional component due to the interaction. The method of evaluating σ_s^2 given above was then no longer satisfactory. It was possible, however, to estimate the size of the component for the sire-year interaction (σ_{st}^2) from the interaction sum of squares calculated for the test of significance given above and to make allowances for its presence in the sum of squares between sires. Heritability was then estimated by

$$\frac{4\ \sigma_s^2}{\sigma_s^2 + \sigma_{st}^2 + \sigma_e^2}$$

Smith (1936) and Hazel *et al.* (1943) used the method of taking expectations of crossproducts in an analysis of covariance to estimate the genetic correlation between two characters. This method was used in the present analysis. The disproportion in the subclass numbers, however, made it necessary to modify the details of finding the covariance components. The difference between the reduction in crossproducts for fitting all constants and the reduction for fitting all but the sire effects supplies an estimate of the covariance component between sires. As shown by Henderson (1953), this crossproduct has an expectation which can be found in the same way as that used in estimating the variance component σ_s^2. In a population mating at random, the covariance component between sires is equal to one-fourth of the covariance between the additive deviations caused by genes in the two characters. The genetic correlation between the two characters was therefore estimated by

$$r_{gg'} = \frac{\text{cov}\ (ss')}{\sigma_s\ \sigma_{s'}}$$

Regression of Daughter on Dam

Since the data contained observations on all characters studied for both daughters and dams, it was possible to determine heritability by doubling the regression of daughter on dam. A method of calculating the genetic correlation between two characters by means of regression analysis was also described by Hazel (1943). This method, which involves measuring the regression of one trait in the daughters on another trait in the dam, was used in finding genetic correlations.

The records taken on the dam introduced a complication. In the first two years, the flock was composed entirely of purchased ewes for which yearling records were not available. For both fleece and body characters, the only records were those taken when the ewes were either two years or five years of age. Hence for the years 1945, 1946, and part of 1947, the regression computed was that of hogget record of the daughter on the mature record of the dam. In some of the 1947, and all of the 1948 data, hogget records were available for the dams.

The data were divided into seven groups so that, within each group, the observations on the dams were comparable in having been taken in the same year and at the same age. This grouping served three purposes. By making the comparisons on a within-group basis, it eliminated the effects which year and age may have had on the records of the dams. It also allowed comparison between heritabilities calculated from the regression of hogget record of the daughter on mature record of the dam with those calculated from hogget record of daughter on hogget record of dam. Further, it did away with the necessity of having to correct the observations on the daughters for differences in age of dam and year in which the observations were made, since all daughters within a group were comparable in these respects.

Correction factors, for type of birth and age at shearing, were used to adjust the records on the daughters for these effects. Correction factors for the dams were calculated to adjust for the effects of the number of lambs born and reared in the year in which the records were taken. These corrections were average differences between ewes with different numbers of lambs born and reared, the differences being computed for each of the four age-year groups of mature ewes.

Of the 576 dams in the study, 82 (or 15%) had twin ewe offspring. In the analysis, measurements on both offspring were included, the measurement of the dam being repeated. Since such a low proportion of the dams have twin offspring, it is unlikely that the estimates of the regressions obtained in this manner would differ greatly from those derived using the method of Kempthorne and Tandon (1953).

RESULTS

The Interaction of Sires and Years

Since the subsequent analysis of the data for the years 1945 to 1947 inclusive depended on the results of the test of significance of the

TABLE 1. TEST OF SIGNIFICANCE OF THE SIRE-YEAR INTERACTION

Character	Mean Squares		Significance
	Interaction	Error	
Quality number	1.023	1.909	N.S.
Staple length	1.836	2.817	N.S.
Fleece quality	6.378	2.137	**
Fleece weight	0.620	0.894	N.S.
Hairiness	0.125	0.080	N.S.
Body type	0.869	1.998	N.S.
Degrees of freedom	12	513	

N.S. = not significant.
** = significant at 1% level.

sire-year interaction, this aspect will be presented first. The mean squares for the interaction term and the error term in the analysis of variance are given in Table 1.

Of the six characters, fleece quality was the only one to show a significant interaction between sires and years. The presence of the interaction implies that the differences between the sires as measured by the means of their progeny were not the same from year to year. Knowing the sizes of the sire effect and of the year effect would be insufficient to allow accurate prediction of the mean of a sire-year group because there is an added component peculiar to the particular combination of sire and year. Thus the merit of a sire measured by its progeny average in one year may be quite different from its merit measured in some other year.

Possible causes of the interaction of these data are:

1. Genuine non-linear interaction between the transmitting ability of the sires and the environmental conditions peculiar to each year. Interaction would occur, for example, if the yearly environments differed in rainfall while the sires differed in the genes they transmitted to their offspring controlling the response of fleece quality to rainfall conditions. If one sire transmitted genes enabling its offspring to produce good fleeces under wet conditions, its progeny average would be high in wet seasons and low in dry seasons, while another, transmitting genes for good fleece quality in dry conditions, would be good in dry years and poor in wet years. The order of apparent merit of the two sires would thus depend on the rainfall conditions of the year in which they were tested.

2. Since each sire was mated to a different group of dams in successive years, there is a possibility that differences between dams could have contributed to the sire-year interaction. For instance, if the genetic average of the mates of a sire was far above the yearly average in one year, and, in the next year, the genetic average of the mates was far below the yearly average, the differences which would be expected between the means of the offspring in the two years would be included in the interaction variance. It is thought that this effect is unlikely to have been important in the present data because the ewes within the flock were assigned to the sires at random. Randomisation would tend to prevent the groups of ewes from averaging genetically far above or below the yearly mean, while any average differences in the dams from year to year were confounded with the year effects and were removed in that source of variation.

There is also a possibility that non-additive gene effects could have contributed to the sire-year interaction. If genes in certain combinations have effects different from their average effects because of dominance and epistasis, the value of a sire as found from mating him to one group of dams may differ considerably from the result found from mating him to some other group. For non-additive gene effects to contribute much to the interaction, the groups of ewes to which a sire was mated in different years would need to be genetically much alike within groups but different within groups. Again, randomisation would tend to prevent the groups of ewes from differing greatly in degree of uniformity within any year.

3. Variations in the standards of scoring may have contributed to the interaction although average differences in the scoring standards were eliminated in the year effects. The fleece quality score is a summary of a number of individual characteristics of wool. If the judges in describing fleece quality had emphasised one of these component characteristics in some years, and in other years had emphasised a different one, and if the sires differed in the genes they transmitted for the two characteristics, variation from this source would contribute to the sire-year interaction.

In an attempt to distinguish between the above possibilities, the interaction effects were computed and are presented in Table 2. The departures from additivity were most marked in the years 1945 and 1946. Sire 10 showed the largest deviation between these two years, the difference being more than 1 3/4 scores, while sire 2 showed a difference of one score.

TABLE 2. VALUES OF THE SIRE-YEAR INTERACTION EFFECTS COMPARED WITH MEAN HAIRINESS FOR EACH SIRE-YEAR GROUP

	1945		1946		1947	
Sire	Effect	Hair	Effect	Hair	Effect	Hair
1	−0.21	0.88	0.56	0.61	−0.35	0.82
2	0.53	0.69	−0.53	0.62	—	—
5	0.14	1.06	−0.42	0.75	0.28	0.77
6	−0.23	0.86	0.31	0.61	−0.08	0.64
7	0.26	0.98	−0.44	0.79	0.18	0.81
8	0.32	0.91	−0.46	0.71	0.14	0.70
10	−0.81	1.15	0.96	0.76	−0.15	0.88
11	—	—	0.01	0.73	−0.01	0.87

The amount of hairiness present in the fleece was known to influence the score given for fleece quality because hairy wool generally shows less crimping and greater tippiness of the staple. It was, therefore, of interest to consider the relationship between the amount of hairiness and the departures from additivity. The mean hairiness of the sire groups corrected for type of birth, age of dam and age at shearing are included in Table 2.

Sire 10, which shows large interaction effects in 1945 and 1946, has the highest mean hairiness in 1945 and second highest in 1946, while sire 2, which also shows large departures from additivity, was consistently low in hairiness in both years. The signs of the interaction effects in 1945 and 1946 suggest that the emphasis placed on hairiness by the judges may have been different in these two years. This variation in emphasis may have been due to the difference in mean hairiness between the two years (0.16 log units). Knowledge of the grading system suggests that there is a threshold value for hairiness below which it is not considered important by the judges. The higher mean hairiness in 1945 would result in a greater proportion of the animals being above this threshold and consequently hairiness would receive more weight in determining fleece quality. Variations in emphasis of this nature offer a tentative explanation for the presence of the interaction in fleece quality.

Further information on the cause of the interaction was obtained by analysing the scores for fleece quality taken prior to lamb shearing to determine whether the sire-year interaction was significant at this age. Only those animals with records at hogget age were included in the analysis which showed that the interaction mean square was just short of significance at the 5% level. This result suggests that whatever caused the interaction had an effect on the lamb score similar to that which it had on the hogget score. If the interaction was caused by varying emphasis placed on hairiness at different times, the means of the scores on the lambs indicated that the direction of emphasis was similar in both the lamb and hogget descriptions of fleece quality. In the same manner, if the interaction was due to non-additive effects between heredity and environment, it seems that the environmental agency concerned acted over the period of growth of both the lamb and hogget fleece.

The data available offered no further possibilities for investigating the cause of the interaction. Summarising, the evidence tentatively points towards variation in the extent to which the judges placed emphasis on hairiness in judging fleece quality as being a contributing factor to the interaction of sires with years.

Phenotypic Correlations among the Fleece and Carcass Traits

The phenotypic correlations among the six characters studied were calculated from the hogget records. Differences due to type of birth and rearing, and age at shearing were removed by the use of correction factors while the effects of years and age of dam were eliminated by calculating the correlations within groups of animals comparable for

TABLE 3. PHENOTYPIC CORRELATIONS AMONG THE CHARACTERS
(based on 628 degrees of freedom)

	Staple Length	Fleece Quality	Fleece Weight	Hairiness	Body Type
Quality number	−0.54**	0.06	−0.33**	−0.11*	−0.08
Staple length		0.20**	0.45**	0.11*	0.16**
Fleece quality			0.15**	−0.24**	0.12**
Fleece weight				0.04	0.17**
Hairiness					−0.06

* and ** denote that the correlation is different from zero at $P < .05$ and $P < 0.1$ respectively.

these factors. The resulting correlation coefficients were combined by the use of the "z" transformation as described by Snedecor (1946). The correlations are presented in Table 3.

The correlation between hairiness and fleece weight agrees with the figures of 0.056 and 0.026 found by Goot (1945) for Romney sheep. The correlation between staple length and fleece weight in this study is higher than the value of 0.34 found by Rae (1946) also with Romneys. No other correlations between fleece characteristics have been reported for Romney sheep.

The results of Spencer *et al.* (1928) working with range Rambouillet sheep varying in age from one to seven years, are generally lower than those presented here. The differences are particularly large in the correlations between fineness of fibre and staple length (—0.0403), staple length and fleece weight (—0.0110) and fineness of fibre and fleece weight (—0.1247). Variations due to age which were not taken into account in this former study may be the cause of the differences between the two sets of results. The correlations between greasy fleece weight and staple length, reported by Pohle and Keller (1943) of 0.38 for Rambouillets, 0.43 for Targhees and 0.42 for Corriedales are similar to that found here for the Romney.

Since Lang (1947) has shown that quality number and crimps per inch are closely correlated, the correlation found in the Romney for staple length and quality number (—0.54) may be compared with the correlation of —0.34 between staple length and crimps per inch found by Morley (1951) for the Australian Merino.

Estimates of Heritability

It has been mentioned that the records available on a number of the dams were those taken when they were five years of age. Since selection of ewes is normally practised at yearling age, the more satisfactory estimate of heritability is that calculated from the regression of hogget measurement of offspring on hogget measurement of dam. Hence it was of interest to examine whether heritabilities calculated from the regression of hogget measurement of daughter on mature measurement of dam differed from those calculated from the regression of hogget measurement of daughter on hogget measurement of dam. The results of the comparison are presented in Table 4.

The differences between the heritabilities were not significant for all characters, the sampling errors being large because of the small number of animals. However, excepting fleece quality, higher values of heritability were found when calculated from the regression of hogget record of daughter on hogget record of dam. The differences were largest for staple length (0.21) and fleece weight (0.20).

The two regressions do not have the same expectation in terms of the additively genetic effects. In particular, a change in either the phenotypic or genic variance with age will cause the heritability to be different unless the change in one is compensated by a change in the other. In Table 4, a comparison of the phenotypic variances of

TABLE 4. COMPARISON OF HERITABILITIES CALCULATED FROM MATURE RECORDS AND FROM HOGGET RECORDS OF DAMS

Character	Mature Records		Hogget Records		Difference
	Heritability	Variance of Dams	Heritability	Variance of Dams	
Quality number	25 ± .08	2.21	.34 ± .13	2.10	− .09 ± .14
Staple length	.29 ± .10	2.79	.50 ± .17	2.53	− .21 ± .19
Fleece quality	.25 ± .10	2.07	.16 ± .12	2.04	.09 ± .15
Fleece weight	.11 ± .07	1.64	.31 ± .16	0.86	− .20 ± .18
Hairiness	.60 ± .08	0.10	.68 ± .12	0.08	− .08 ± .15
Body type	.13 ± .11	2.27	.17 ± .13	2.16	− .04 ± .16
Degrees of freedom	477		163		

measurements indicates little change from one age to the other for all characters except fleece weight. For these characters, since the differences between the estimates of heritability at the two ages were not significant and since the phenotypic variances were similar in size, the two estimates were combined by pooling the crossproducts and sums of squares. The combined estimates are presented in Table 5.

TABLE 5. HERITABILITIES COMPUTED FROM THE REGRESSION OF DAUGHTER ON DAM

(640 degrees of freedom)

Character	Heritability
Quality number	0.27 ± .05
Staple length	0.35 ± .07
Fleece quality	0.22 ± .07
Hairiness	0.63 ± .06
Body type	0.14 ± .07

The variance of fleece weight measured at maturity is almost twice as large as the variance of hogget fleece weight. The coefficients of variation are, however, similar in size (16.9% for mature fleece weight and 16.0% for hogget fleece weight). The consistency of the coefficient of variation when the means vary suggests that age as a cause of variation in fleece weight acts in a multiplicative manner rather than additively. The large difference in phenotypic variance and the difference between the two estimates of heritability for fleece weight indicate that the values for the genic and environmental variances are not comparable; hence the two estimates were not combined.

Heritabilities as computed by the paternal half-sib correlation from the variance components between sires and for error are presented in Table 6. For fleece quality, where the interaction of sires with years was significant, it was also necessary to estimate the interaction component of variance and to make allowance for its presence in the sum of squares between sires.

TABLE 6. HERITABILITIES COMPUTED FROM PATERNAL HALF-SIB CORRELATIONS

Character	Mean Square Sires	Mean Square Error	σ_s^2	σ_{st}^2	Heritability
Quality number	10.13	1.81	0.242	—	0.47
Staple length	14.88	2.63	0.356	—	0.48
Fleece quality	4.34	1.96	0.069	0.210	0.12
Fleece weight	3.38	0.85	0.074	—	0.32
Hairiness	0.317	0.077	0.007	—	0.34
Body type	4.52	1.78	0.080	—	0.17
Degrees of freedom	17	664			

The values of heritability computed from the paternal half-sib correlation are of limited reliability since only 17 degrees of freedom are available on which to base an estimate of the variance among genetic values of the sires. By chance, the sires could have been more nearly alike or more diverse than was typical of the population from which they were drawn. However, with the exception of hairiness and, to a lesser degree, quality number, the estimates in Table 6 agree closely with those in Table 5. No explanation of the difference between the two estimates for hairiness can be offered.

TABLE 7. COMPARISON OF GENETIC CORRELATIONS WITH FLEECE WEIGHT CALCULATED FROM THE MATURE RECORDS OF DAMS WITH THOSE CALCULATED FROM HOGGET RECORDS OF DAMS

Character	Mature Records		Hogget Records	
	Estimate	Standard Error	Estimate	Standard Error
Quality number	−0.47	0.22	−0.49	0.39
Length	0.26	0.21	0.21	0.44
Fleece quality	0.09	0.26	−0.16	0.39
Hairiness	0.27	0.17	0.38	0.30
Body type	0.05	0.35	0.09	0.48
Degrees of freedom	477		163	

Genetic Correlations among Fleece and Carcass Traits

The method of estimating genetic correlations by measuring the regression of one trait for the daughter on another trait for the dam has been described. Since it was shown that the variance of the mature fleece weights of the dams differed from that of the dams with fleece weights at hogget age, the genetic correlations of the other traits with fleece weight were calculated separately for the two groups. The results are presented in Table 7. The two sets of correlations show close agreement in size; hence they were combined by weighting each estimate by the reciprocal of its variance to give the values shown in Table 8.

TABLE 8. GENETIC CORRELATIONS AMONG THE CHARACTERS

	Staple Length	Fleece Quality	Fleece Weight	Hairiness	Body Type
uality number	−0.73 ± 0.16	0.21 ± 0.17	−0.47 ± 0.19	−0.30 ± 0.11	0.08 ± 0.22
taple length		0.13 ± 0.19	0.25 ± 0.19	0.41 ± 0.12	0.21 ± 0.31
leece quality			0.08 ± 0.22	−0.12 ± 0.12	0.48 ± 0.32
leece weight				0.28 ± 0.16	0.06 ± 0.30
airiness					−0.30 ± 0.25

Genetic correlations were also obtained from estimates of the covariance components between sires for each pair of traits. As with estimates of variance components, the accuracy of the estimated covariance components depends in a large measure on the number of sires included in the analysis. Since the estimates were determined from the data for the years 1945 to 1947, only 12 degrees of freedom for sires were available for estimating the genetic covariances.

TABLE 9. GENETIC CORRELATIONS AMONG THE CHARACTERS AS ESTIMATED FROM THE COVARIANCE COMPONENTS BETWEEN SIRES

	Staple Length	Fleece Quality	Fleece Weight	Hairiness	Body Type
Quality number	−0.76	−0.41	−0.62	0.07	−0.10
Staple length		0.74	0.60	0.34	0.30
Fleece quality			0.27	−0.29	0.49
Fleece weight				−0.28	0.00
Hairiness					−0.34

The accuracy of these estimates is likely to be lower than those derived from the regression of offspring on parent. However, they serve as a check both on the sign and magnitude of the values in Table 8. Because of this lower accuracy, it was considered to be not worth the extra computation to take into account any contribution from the sire-year interaction to the covariance between sires of fleece quality with other traits.

The agreement between the values in Tables 8 and 9 is reasonably good. A notable difference in sign occurs in the correlation between quality number and fleece quality where the paternal half-sib method shows a correlation of −0.41 compared with 0.21 in Table 8. The two correlations between staple length and fleece quality also differ while a difference in sign exists between the two estimates for the correlation between fleece weight and hairiness. Since the sampling errors are so large, it is unlikely that any importance should be attached to these differences.

DISCUSSION

Genetic Variation

An estimate of a genetic parameter such as heritability applies to the particular population from which the data were drawn and, in theory, may vary from one population to another because of factors which may cause differences in either the genetic or environmental variances. Application of the results in this study to other flocks of Romney sheep thus involves the assumption that the phenotypic and genetic relationships are similar to those found in the present flock. A comparison of the heritabilities with previous results as reviewed by Rae (1956) shows them to be similar and consequently there is some justification for the assumption needed to generalise the present results.

In previous studies, McMahon (1943) and Rae (1948) agreed in quoting a range of values of heritability from 0.10 to 0.15 for greasy fleece weight, while Rasmussen (1942) working with Romney crossbreds in Canada reported a range of 0.10 to 0.16. In the present study, heritability of 0.11 as determined by regression of offspring on mature record of the dam agrees with the earlier figures, but the values of 0.32, found by paternal half-sib correlation and 0.31 by the regression of offspring on hogget record of the dam are higher than the other results. Since the accuracy of the last two results is low, the difference between them and earlier results may well be due to sampling errors. The possible reasons for the higher values of heritability of greasy fleece weight in fine-wool breeds have been discussed by Morley (1951) and Rae (1956).

For fleece quality, McMahon (1943) reported a heritability of 0.14 in the Romney, the same figure also being found by Rae (1948). The present results of 0.22 and 0.12 are in general agreement with these earlier results. The presence of interaction between sires and years in fleece quality requires consideration when interpreting the heritability for this trait. Possible causes of the interaction have been stated but the present data do not indicate decisively which of these causes was important in producing the interaction. Knowledge of the cause of the interaction would be useful in deciding on methods to take account of its effects on selection. If the cause is a real interaction between heredity of the sire and the environmental conditions prevailing in particular years, it does not seem possible to take advantage of the specific combinations of heredity and environment in a selection programme because the breeder is unable to predict what the subsequent yearly conditions will be. In this case, the presence of the interaction acts mainly as a source of inaccuracy in estimating the breeding value of the animal and thus reduces the efficiency of selection. If the interaction is caused by "nicking" effects of the sire with particular groups of dams, it still results in less efficient selection, although modification of the breeding programme to emphasise line-breeding and family selection may allow advantage to be taken of these specific effects. Finally, if the interaction results from varying the emphasis given to different component characteristics in describing fleece quality, improving the scoring system may do much to make the relationship additive. It would be necessary to decide on the degree to which variations in the component characters are to be scored up or down, thus defining in more detail the series of grades to be used in the description.

From the standpoint of selection, presence of the interaction whatever may be its cause leads to lower accuracy in judging the breeding value of an animal. Permanent genetic gain comes from the action of selection on the additive genetic effects, although some temporary change may occur if the non-additive effects are correlated between parent and offspring. In the estimation of heritability, it is therefore desirable to exclude the variance due to non-additive effects from the numerator, while still retaining this variance in the denominator of the estimate. It would seem that this has been achieved in the estimate derived from the paternal half-sib correlation.

The presence of the interaction between sires and years gives some reason for believing that a similar interaction may exist between the dams and years, though it would be extremely difficult to obtain data in sheep to show it. If any interaction effects are present and correlated between dam and offspring, heritability found by the regression of daughter on dam will be over-estimated. There is some reason for believing that this correlation is not likely to be large, because in all cases measurements on the dam were taken in a different year from the measurements on the daughter, thus giving the opportunity for deviations in one direction to be cancelled by deviations in the other direction. Comparison of the heritability from the paternal half-sib correlation (0.12) with the two results from the regression of daughter on dam (0.25 and 0.16) suggests that any contribution from the interaction is not large.

Heritability of hairiness is high in the Romney breed. The present result is in agreement with previous results and is supported by the rapid response of this character to selection. The heritability for quality number found by regression of daughter on dam (0.27) is lower than those previously reported by Rae (1948) and McMahon (1943) while the heritability of staple length is somewhat higher than earlier results. No reason for these differences can be suggested.

All results reported to the present time agree in placing body type as a weakly heritable character. It is well known that body form is influenced considerably by nutrition and other environmental factors. Moreover, desirable conformation is a complex character, in which symmetry and balance of the body parts play an important part. Stonaker and Lush (1942) in discussing heritability of conformation in swine, have suggested that the highest scoring animal may be an intermediate in many of the visible characters making up the type score. For characters where this is the case, many of the genes will act epistatically in their effects on body-type score even though they act additively on the component anatomical parts of the body. Heritability may thus be low because much of the variation is epistatic in nature. Moreover, body type is such a complex trait that no series of body measurements has yet been found to replace the subjective method of scoring the animal by comparison with a mental standard. Errors in scoring, in so far as they were random, contribute to the environmental variance. Hence, the low value of heritability may be accounted for in part by the inaccuracy of the scoring of body type.

The results show that progress in improving body type, fleece weight, and fleece quality from individual selection will be slow. Sufficient genetic variability is present in the remaining traits to give a more rapid response to selection. The pattern of genetic correlations must, however, be considered to see to what extent they are a factor in limiting improvement for selection.

Genetic Covariation

The accuracy of the estimates of genetic correlations is far from satisfactory, as is shown by the size of the variances calculated for the results from regression of daughter on dam. Since the genetic correlation is computed as a function of four covariances each of which has its own sampling error, large sampling variances are to be expected. Using present methods, considerably more observations than were available for the present investigation are required to reduce the standard errors to a level where confidence can be placed in the estimates. For most of the correlations, the standard error was about 0.20. Its size depends in a rather complex manner upon the number of daughter-dam pairs and the expected size of the various covariances between the two traits of dam and of offspring. Considering only the number of daughter-dam pairs, four times the present number, or about 2500 pairs would be required to reduce the standard error by one half. Nevertheless, in the present case, the agreement of the results from regression of daughters on dam with those derived from the covariance components between sires, gives one a little more confidence in the values obtained.

No other estimates of genetic correlations between fleece and body characters are available in the New Zealand Romney for comparison with the present results. Morley (1955a, b), however, has reported a number of genetic correlations in the Australian Merino, some of which are comparable with the present data. In general, the agreement between the two sets of results is good.

Although the actual figures for the genetic correlations are not accurate enough to allow wide conclusions to be drawn from them, the general pattern of the relationships can be sketched. Selection for increased greasy fleece weight will produce correlated responses in the direction of coarser wool, slightly greater staple length, no change in fleece quality and body type, and an increase in hairiness. Selection for greater staple length will be accompanied by coarser, heavier, and more hairy fleeces with not much alteration in fleece quality and body type. Selection against hairiness will result in fleeces being finer, shorter in staple length, and lighter but slightly better in fleece quality. Some improvement in body type is to be expected with decreased hairiness. The main genetic antagonisms are between quality number and staple length, quality number and fleece weight, and hairiness and fleece weight.

The extent to which these antagonisms will interfere with improvement depends to a considerable extent on the relative economic importance of fleece weight, quality number, and hairiness. If it is desired to increase fleece weight without changing quality number, then the rate of progress in fleece weight is only about 55% of that which would be achieved if selection were for fleece weight alone. The corresponding rate of progress if it is desired to hold hairiness constant is only 30% of that achieved if fleece weight were the only character selected. Rae (1954) has shown that fleece weight has a relative economic importance which is much greater than either hairiness or quality number. In practice, this would imply that some small increase in hairiness may be justifiable in order to gain greater efficiency in selection for the more remunerative character fleece weight. The situation with regard to quality number is more confused because there is some evidence that for a given environment there may be an optimum value for quality number which is in the intermediate range of quality numbers. This aspect requires further analysis.

While the above pattern defines the statistical properties of the population in its response to selection, it supplies little information on the causes of these correlated responses. The agreement of the present genetic correlations between fleece weight, staple length, and fineness with those of Morley (1955a, b) suggests that this set of relationships is of fundamental significance in the physiology of wool production. In addition, the picture of genetic correlations among fleece characters is similar to the associations of traits which are characteristic of different breeds. Generally speaking, long staple, coarse fibre and, partly as a consequence, heavy fleece weight go together. This complex of characters is generally but not invariably associated with a high amount of hairiness.

The genetic mechanisms which may underlie the above correlated responses have been reviewed by Morley (1951) and Rae (1951) and will not be elaborated here. In the present case, in so far as pleiotropy is a common cause of genetic correlations, it is interesting to note that the information which has been built up on the action of the N gene is pertinent to the discussion of the cause of the genetic correlations of hairiness with fleece weight and with body type. The most obvious effect of the *N* gene (Dry 1955) is to produce a high abundance of halo hairs in the birth coat of the lamb. Investigation of the mode of action of this gene has shown that its main effect is to increase the vigour of the primary follicles (Fraser *et al.* 1954). Thus the *N* gene can be regarded as an example of the type of gene that could give rise to the genetic correlation between fleece weight and hairiness. In addition, it has been shown that, under certain circumstances, the *N* gene has a pleiotropic effect on the rate of body growth and hence on the body conformation of the sheep which carry it (Cockrem, pers. comm.). The presence of genes with a similar but not as extreme effect as the *N* gene would lead to the negative genetic correlation of −0.30 between hairiness and body type. However, much more information is required about the basic functioning of the fibre follicle and its relationship to body growth before any such explanation can be substantiated.

ACKNOWLEDGEMENTS

The author is indebted to Mr. R. A. Barton of Massey Agricultural College for his valuable assistance in collecting the data on which this investigation was based and to Mr. W. R. R. Hewitt and his staff for their co-operation in the management of the flock. The writer also wishes to express his appreciation of the counsel given in the course of this investigation by Dr. L. N. Hazel, Dr. J. L. Lush, Dr. J. W. Gowen, and Mr. O. Kempthorne of Iowa State College. Financial assistance from the Department of Scientific and Industrial Research is gratefully acknowledged.

REFERENCES

BARTON, R. A. 1947: *Proc. 10th annual Mtg. Sheepfmrs. Massey Agric. Coll.* 1947 p. 126.
CLARKE, E. A.; BARTON, R. A.; WILSON, G. S. 1953: "The Effect of Highly Improved and Topdressed Pastures on Thrift and Production of Sheep." Massey Agricultural College, Palmerston North.
DRY, F. W. 1955: *Aust. J. agric. Res. 6:* 725.
FRASER, A. S.; ROSS, J. M.; WRIGHT, G. M. 1954: *Ibid. 5:* 490.
GOOT, H. 1945a: *N.Z. J. Sci. Tech. A27:* 45.
——— 1945b: *Ibid. A27:* 357.
——— 1946 : *Ibid. A28:* 80.
——— 1952 : *Ibid. A34:* 106.
HAZEL, L. N. 1943: *Genetics 28:* 476.
——— 1946: *Biometrics Bull. 2:* 21.
HAZEL, L. N.; BAKER, M. L.; REINMILLER, C. F. 1943: *J. Anim. Sci. 2:* 118.
HENDERSON, A. E.; MCMAHON, P. R. 1947: *N.Z. J. Sci. Tech. A29:* 22.
HENDERSON, C. R. 1953: *Biometrics 9:* 276.
KEMPTHORNE, O. 1952: "The Design and Analysis of Experiments." Wiley, New York.
KEMPTHORNE, O.; TANDON, O. B. 1953: *Biometrics 9:* 90.
LANG, W. R. 1947: *J. Textile Inst. 38:* T257.
LUSH, J. L. 1941: *Proc. Amer. Soc. Anim. Prod. 1940* p. 293.
——— 1949: *Hereditas, Suppl. Vol., 1949:* 356.
MCMAHON, P. R. 1937: *J. Textile Inst. 28:* T349.
——— 1943: *Proc. N.Z. Soc. Anim. Prod. 1943.*
——— 1946: *Proc. N.Z. Soc. Anim. Prod. 1946,* p. 80.

MORLEY, F. H. W. 1951: *N.S.W. Dep. Agric. Sci. Bull. 73.*
——— 1955a: *Aust. J. agric. Res. 6:* 77.
——— 1955b: *Ibid. 6:* 873.
POHLE, E. M.; KELLER, H. R. 1943: *J. Anim. Sci. 2:* 33.
RAE, A. L. 1946: *Univ. of N.Z. Thesis,* Massey Agric. Coll. Library.
——— 1948: *Proc. N.Z. Soc. Anim. Prod. 1948*, p. 97.
——— 1951: *Ibid. 11:* 104.
——— 1954: *Sheepfmg. annu. Massey Agric. Coll. 1954,* p. 143.
——— 1956: *Advances in Genetics 8:* 189.
RASMUSSEN, K. 1942: *Sci. Agric. 23:* 104.
SERRA, J. A. 1948: *Publ. Jta nac. Prod. pecuar. Lisboa A No. 1.*
SMITH, F. 1936: *Ann. Eugen., Lond. 7:* 240.
SNEDECOR, G. W. 1946: "Statistical Methods Applied to Experiments in Agriculture and Biology". 4th ed. Iowa State College Press, Ames, Iowa.
SPENCER, D. A.; HARDY, J. I.; BRANDON, M. J. 1928: *U.S. Dep. Agric. Tech. Bull. 85.*
STEVENS, P. G. 1948: *Canterbury [N.Z.] Agric. Coll. Tech. Bull. 2.*
STONAKER, H. H.; LUSH, J. L. 1942: *J. Anim. Sci. 1:* 99.
WRIGHT, G. M.; STEVENS, P. G. 1953: *N.Z. J. Sci. Tech. A34:* 430.

NEW ZEALAND
JOURNAL OF SCIENCE

Volume 1 March 1958, No. 1.

CONTENTS
(provisional)

The Estimation of Missing Values in Factorial Experiments. *G. M. Wright*

The Dichromate—Lignosulphonate Reaction and Its Potential Use in Chemical Grouting. *Allette Weston* and *R. A. Kennerley.*

Low-temperature Adsorption of Various Gases by Soils. *K. S. Birrell* and *R. Q. Packard.*

Bacteriological Survey of Auckland Harbours. Part 5. *G. M. Wallace, Lorraine E. Newman,* and *Jacqueline L. Jerrome.*

Nutritional Studies of *Phytophthora cactorum* (Leb. and Cohn) Schroet. Part 1. *T. Christie.*

Corrosion of Mild Steel in Some New Zealand Soils. *H. R. Penhale.*

Optimum Arrays for Direction Finding. *N. F. Barber.*

The Steam Distillation of Volatile Fatty Acids. *R. O. Weenink.*

Sulphur Isotopic Variations in Nature. Part 4. *J. R. Hulston* and *B. W. Shilton.*

CONTENTS

PAGE

Effects of Differences in Light Intensity and Temperature on the Anatomy and Development of Leaves of *Lolium perenne* and *Paspalum dilatatum*. *K. J. Mitchell* and *Kathleen Soper* 1

A Characteristic Filamentous Bacterial Rumen Organism Indicative of a Sustained Low Ruminal Ammonia-nitrogen Level. *N. D. Jamieson* and *T. M. Loftus* 17

Resistance to Yellow-leaf Disease in *Phormium tenax* Forst., and Observations on Delayed Expression of Symptoms. *W. R. Boyce* 31

Effect of "Metasystox" on Cabbage Aphid (*Brevicoryne brassicae* (*L.*)). *A. D. Lowe* 37

Development of Streptomycin-resistant Variants of *Pseudomonas syringae* van Hall in Culture and in Peach Seedlings. *M. H. Dye* 44

Pasture Responses to Phosphatic Fertilizers. *J. Karlovsky* 51

The Light Regime within Pastures. *K. J. Mitchell* and *D. M. Calder* 61

The Effect of Stilbestrol Implants upon the Growth and Fattening of Romney Wether Lambs. *L. J. Lambourne* 69

Note on Transmission of Apple Mosaic by Natural Root Grafting. *J. A. Hunter*, *E. E. Chamberlain*, and *J. D. Atkinson* 80

Thyroid Follicular Cell Height. *R. E. Munford* and *A. H. Kirton* 83

Genetic and Environmental Components of the Productivity of Perennial Ryegrass (*Lolium perenne* L.). *S. O. Fejer* 86

Genetic Variation in New Zealand Romney Marsh Sheep. *A. L. Rae* 104

SUBSCRIPTIONS. Subscriptions will be accepted only on advance payment and only for a full calendar year. Price: 30s. per annum; single copy 5s.; post free (surface mail). Published in alternate months, starting February. Subscriptions should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 8018, Wellington, New Zealand. Cheques should be made payable to the Department of Scientific and Industrial Research, and if drawn on banks outside Wellington, must have exchange added. Subscribers are requested to give their name and full postal address.

This journal may also be ordered through The High Commissioner for New Zealand, 415 Strand, London W.C.2, or through any bookseller.

Printed by Coulls Somerville Wilkie Ltd.,
Wellington, New Zealand.

New Zealand Journal
of
Agricultural Research

VOLUME I No. 2

APRIL 1958

COMMONWEALTH MYCOLOGICAL INSTITUTE
21 MAY 1958

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind, however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. 4¼ × 7 in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is 4¼ × 7 in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be, shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation, etc., of references, see examples in this issue.

NEW ZEALAND JOURNAL
of
AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. A. Black.

Vol. 1. April 1958 Number 2

THE EFFECT OF LENGTH OF PASTURES ON COBALT-DEFICIENCY DISEASE IN LAMBS

By E. D. Andrews[1], B. J. Stephenson[1], J. P. Anderson[2], and W. C. Faithful[3]

(*Received for publication, 8 November 1957*)

Summary

Preliminary investigations in 1951 at the Winton Experimental Farm, Southland, on a soil type classified as Otapiri silt loam showed that cobalt-deficiency disease was much more marked among lambs on one particular paddock in which pasture was long, than on adjacent paddocks where pastures were short. Experiments to compare the effects on cobalt deficiency of long and short pastures, as determined by light and heavy grazing respectively, were made during the next two seasons. Interpretation of results was based on evidence from the analyses of pasture and liver samples for cobalt content, weight changes of lambs, and weight responses of lambs to cobalt dosing.

It was found that longer pastures could not account fully for the more marked unthriftiness of lambs in the one particular paddock. Nevertheless, short heavily-grazed pastures appeared to reduce the severity of cobalt-deficiency disease among lambs in the one particular paddock, and prevented it entirely in other more mildly cobalt-deficient paddocks. It is considered that increased cobalt intake from soil-contaminated pastures offers the most probable explanation of the protection afforded by heavy grazing.

It was estimated that within a range of conditions similar to those of the trials the minimal level of pasture-associated cobalt needed by lambs is about 0.11 p.p.m.

Evidence from other farms on Otapiri silt loam coupled with results obtained at the Winton Experimental Farm shows that this soil type can be classified as marginally cobalt-deficient.

Preliminary Investigations

Since 1946, Wallaceville workers have routinely used a slightly simplified modification of the criteria first proposed by McNaught (1949)

---

1. Animal Research Station, Department of Agriculture, Wallaceville.
2. Formerly Department of Agriculture, Invercargill. Present address: Timpanys No. 5 R.D., Invercargill.
3. Department of Agriculture, Invercargill.

N.Z. J. agric. Res, 1: 125-139.

for assisting in the diagnosis of cobalt-deficiency disease in cattle and sheep. These criteria are based on the cobalt content of livers and for lambs are as follows:

Low — <0.07 p.p.m. D.M.
Borderline — 0.07–0.09 p.p.m. D.M.
Normal — $\geqslant 0.10$ p.p.m. D.M.

During February 1951, specimens from 3 unthrifty lambs which had been confined to one particular paddock (No. 7, Fig. 1) at the Winton Experimental Farm, Southland, were submitted to Wallaceville for diagnostic examination. The livers contained 0.02, 0.03 and 0.04 p.p.m. cobalt. Two pasture samples taken from paddock 7 on 5 April contained 0.07 and 0.08 p.p.m. cobalt, amounts which could be consistent with cobalt-deficiency disease in lambs (McNaught and Paul, 1939). Analytical results, therefore, suggested cobalt deficiency.

Previously, observations had indicated that lambs born about September 1950, and since then confined to paddock 7, started to deteriorate about 3 months later. By April 1951, a number had died and most of the remainder were unthrifty. By contrast, lambs in adjacent paddocks were in considerably better condition at this time. For example those in paddock 10 (Fig. 1) averaged 79 lb, whereas the mean weight of survivors in paddock 7 was only 59 lb.

On 19 April 1951, lambs in paddock 7 were divided into two groups of approximately equal average body weight. Each group contained 12 lambs. One group served as a control and lambs in the other each received 4 mg cobalt as an aqueous solution of cobalt sulphate at weekly intervals. After 7 weeks the lambs given cobalt had gained a mean of 12.5 lb, whereas the mean gain shown by the control group was only 3.9 lb. The 8.6 lb difference in favour of the cobalt-dosed group was statistically significant ($P < 0.05$).

While it was now clear that cobalt deficiency was a factor responsible for unthrifty lambs in paddock 7, it was not certain that the pasture cobalt available to lambs in adjacent paddocks was fully adequate for normal growth. Of more immediate interest, however, was the marked difference in thrift in favour of the animals in these latter paddocks.

Data compiled by the New Zealand Soil Bureau showed that the whole of the Winton Experimental Farm was situated within an area of river flats provisionally classified as "Otapiri silt loam" (Mr. E. J. B. Cutler, pers. comm.). A more detailed survey of the farm carried out by Mr. Cutler did not reveal any obvious soil differences between paddock 7 and adjacent paddocks.

In an early report, Dixon (1936) discussed observations which suggested that short pastures were beneficial in controlling cobalt-deficiency disease in lambs at Morton Mains, Southland. At the Winton Experimental Farm no unthriftiness in paddock 7 had been noted in early 1949 and early 1950 when the paddock was stocked at the rate of 9 lambs to the acre. In the 1950–51 season the paddock had been stocked initially at the rate of 6½ ewes and 6½ lambs to the acre but because of deaths the number of lambs between February and June 1951 averaged about 5 to the acre. Throughout this period pastures remained long. Adjacent paddocks were stocked at a rate of 8½ lambs to the acre, and pastures were kept short. Hence the Winton observations gave some support to the hypothesis that short pastures tend to prevent cobalt deficiency in lambs.

In the present paper we describe experiments carried out during the 1951–52 and 1952–53 seasons to test this hypothesis. In addition an estimate was made of the minimal average level of pasture-associated cobalt needed to satisfy the cobalt requirement of lambs.

Experimental

Trial Paddocks

Three paddocks were used; paddock 7 (5 acres) and the adjacent paddocks 10 and 11 (each 1⅓ acres). So far as could be ascertained the history of these paddocks had been as follows:

Paddock 7 was ploughed and sown with oats in 1947. The paddock probably received lime at the rate of one ton per acre and superphosphate at the rate of 2 cwt per acre, and was resown either with pasture or pasture plus oats in 1948. The pasture seed mixture seems to have been perennial ryegrass, timothy, dogstail, and white clover. Thereafter, annual lime and superphosphate topdressing rates were ½ ton and 2 cwt per acre respectively. In October 1948 the paddock was temporarily divided into two equal parts. At this time, and in the following two years, one half was topdressed with copper sulphate at the rate of 5 lb per acre. As judged by carcass weights in January and March 1949, and in January 1950, there was no evidence that copper sulphate had affected the growth of lambs.

Before 1946 paddocks 10 and 11 were used as a single area. In October 1936 the area was sown with a mixture of perennial ryegrass and white clover. Before sowing the area was topdressed with lime at the rate of one ton per acre, and superphosphate at the rate of 2½ cwt per acre was applied with the grass seed. Thereafter, until 1946, annual applications of lime and superphosphate were probably at the rates of ½ ton and 2 cwt per acre respectively. In 1946 the area was permanently divided into the two present paddocks. Subsequently both paddocks were topdressed with lime at the rate of 2 tons per acre in June 1946 and one ton per acre in March 1948. Paddock 10 was topdressed with reverted superphosphate at the rate of 3 cwt per acre in April 1947, and the same fertiliser at the same rate was applied each subsequent year up to and including 1950. Paddock 11 was topdressed with serpentine superphosphate at 3 cwt per acre in April 1947 and the same fertiliser at the same rate was applied each subsequent year up to and including 1950. As judged by the numbers of lambs fattened in paddocks 10 and 11 during the 1947 to 1950 period, there was no evidence that the different kinds of superphosphate used resulted in a difference in lamb thrift.

Thus, it appeared that there had been little difference in previous treatments between paddocks 10 and 11. The main difference in history between paddock 7 and the other two paddocks was that the former had been cultivated, cropped, and regrassed relatively recently, whereas paddocks 10 and 11 had been continuously in pasture since 1936. At the start of the present trials, however, no marked visible differences in pasture composition of the three paddocks were apparent.

Layout of Paddocks. The layout of experimental paddocks and grazing treatments during the two seasons are shown in Fig. 1.

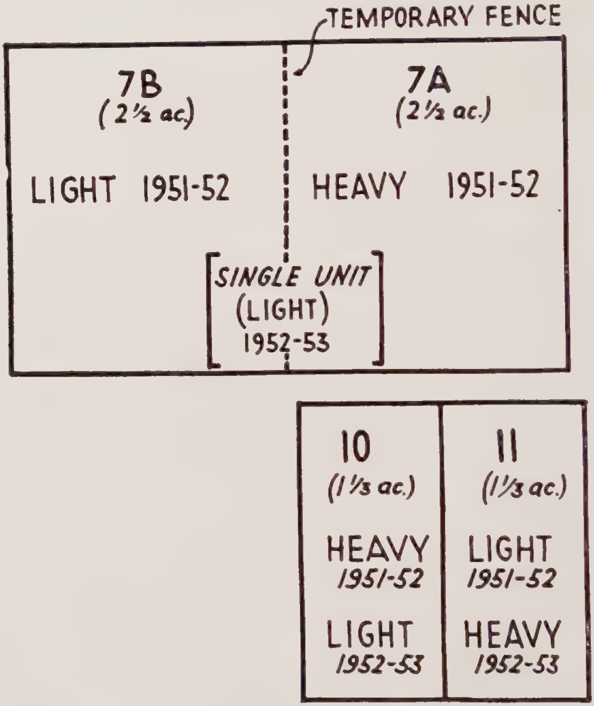

TEMPORARY FENCE
7B
(2½ ac.)
LIGHT 1951-52
7A
(2½ ac.)
HEAVY 1951-52
SINGLE UNIT
(LIGHT)
1952-53
10
(1⅓ ac.)
HEAVY
1951-52
LIGHT
1952-53
11
(1⅓ ac.)
LIGHT
1951-52
HEAVY
1952-53

Fig. 1.—Experimental paddocks and grazing treatments.

For the 1951–52 trials paddock 7 was divided into two equal areas (designated as paddocks 7A and 7B) by a temporary fence and was used as one experimental block. Paddocks 10 and 11 served to replicate grazing treatments as a second experimental block. Paddocks were assigned to treatments as follows:—

Light grazing : Nos. 7B and 11.
Heavy grazing : Nos. 7A and 10.

Later in the season the temporary fence dividing paddock 7 was removed and the area grazed as a single unit for other experimental purposes.

For 1952–53 season grazing treatments in paddocks 10 and 11 were reversed so as to obviate possible effects of other unknown differences between the paddocks.

Light grazing : No. 10.
Heavy grazing : No. 11.

During this season paddock 7 was grazed as a single unit for other experimental purposes.

Pasture Samples for Cobalt Analyses. During both seasons, pasture samples were taken from experimental paddocks at monthly intervals, from November until May inclusive, and were analysed for cobalt content.

At the Winton Experimental Farm, rectangular wire-netting frames, each enclosing $\frac{1}{200}$ acre, are routinely used for pasture-production measurements. At the start of the 1952–53 trials, four of these were placed in paddock 7, and two each in paddocks 10 and 11. At the times when pastures in each paddock were sampled, a composite sample was also taken from within the frames. After each sampling, frames were moved to another part of the paddock. In this way it was possible, under conditions of both light and heavy grazing, to make contemporaneous comparisons between cobalt figures for continuously grazed pasture and for pasture not subjected during the previous month to grazing and trampling by stock.

Management of Grazing Trials

In the lightly grazed paddocks the aim was to keep pastures as long and as even as possible (about 3 5 in.) and in the heavily grazed paddocks as short as possible (about 1 in.) without reducing experimental animals to starvation levels. From previous experience an estimate was made of the number of experimental animals to the acre which should be assigned to each treatment in order to achieve the desired result. During both seasons Romney × Southdown crossbred lambs were used and were allotted to their respective paddocks at birth, that is from about September of each year. From October of each year the effects of treatments were determined by weighing the lambs at intervals until the trials were concluded. Lambs were weaned at about 4 months of age when ewes were removed from experimental paddocks.

1951–52 Season. During this season, lightly grazed paddocks were stocked initially at the rate of 1½ ewes and 6 lambs per acre, and heavily grazed paddocks at the rate of 7 ewes and 9 lambs per acre until weaning. Thereafter dry sheep were on occasion and where required introduced into experimental paddocks to assist in maintaining pastures at the desired lengths. Even so, this proved difficult. Thus, the length of pasture in paddock 7A remained appreciably longer than that of its replicate (No. 10) and in the lightly grazed paddocks pastures tended to become "clumpy" towards the end of the trial. Nevertheless the general objective of maintaining long pastures under light grazing conditions and short pastures under heavy grazing conditions was achieved.

During March 1952 one lamb died in paddock 7B and three in paddock 7A. Their livers were secured for cobalt analyses. In paddock 7B and 7A, light and heavy grazing treatments continued until 2 April 1952. Thereafter, lambs were divided into control, cobalt-dosed, and vitamin B_{12}-injected groups, and continued to graze paddock 7 as a single unit until 4 June. Details of this experiment have been reported elsewhere (Anderson and Andrews 1952).

In paddocks 10 and 11, grazing treatments continued until 24 April when shortage of feed in paddock 10 caused this part of the trial to be terminated. Two livers for cobalt analyses were secured from lambs in paddock 11 and one from a lamb in paddock 10.

1952–53 Season. Although, during the 1952–53 season, paddock 7 was not included as part of the trial of light versus heavy grazing, lamb weights and pasture-cobalt data were available for comparison with similar data for the other two paddocks. Paddock 7 was stocked at the rate of 5½ ewes and 8 lambs to the acre. Subsequent experience showed that this was a light stocking rate for this paddock in the sense that pastures remained long. On 26 February 1953, lambs in paddock 7 were divided into control, cobalt-dosed, and other groups in which vitamin B_{12} was given either orally or by injection. Treatments continued until 10 June, and livers of 4 control lambs that died within this period were secured for cobalt analyses. Details of the experiment have been reported elsewhere (Andrews and Anderson, 1954).

As a result of experience gained in the 1951–52 trial, the number of ewes and lambs assigned to each of the two grazing treatments during the 1952–53 season was increased. The lightly grazed paddock (now No. 10) was stocked at the rate of 6 ewes and 7½ lambs to the acre, and the heavily grazed paddock (No. 11) at the rate of 9 ewes and 11 lambs to the acre. During this season the desired lengths of pasture were better secured, without the periodical introduction of dry sheep after removal of the ewes. In paddock 10 (and in No. 7) the pasture remained at about 3–5 inches in length and was fairly evenly grazed, while that in paddock 11 varied little from the 1 inch length desired. It was possible to carry on the trials of light versus heavy grazing (paddocks 10 and 11) for longer than in the previous season, so that on 22 April 1953 lambs in each paddock were divided into two sub-groups. In each paddock one sub-group served as a control while in the other lambs each received cobalt at the rate of 7 mg a week, until the trial was concluded. At this time two non-dosed lambs from each paddock were killed and the livers taken for cobalt analyses.

RESULTS

Cobalt Contents of Pasture Samples

Table 1 shows results of cobalt analyses of pasture samples taken from experimental paddocks during the two seasons. As discussed later, cobalt-deficient lambs began to lose weight in both seasons at about, or before, February. For this reason, mean cobalt figures for pasture samples from February to May inclusive are given as well as means for samples from November to May inclusive.

Differences Between Seasons. During the 1951–52 season, the cobalt contents of pasture samples from each paddock were in general high from November to January. Thereafter, values fell and remained

TABLE 1. COBALT CONTENT (p.p.m. D.M.) OF PASTURE SAMPLES FROM EXPERIMENTAL PADDOCKS

Season	1951–52				1952–53					
Paddock No.	7B	7A	11	10	7		10		11	
Grazing Treatment	Light (1)	Heavy (1)	Light (2)	Heavy (2)	Light		Light		Heavy	
Cobalt status of lambs (3)	Deficient	Deficient	Deficient	Sufficient	Deficient		Deficient		Sufficient	
						(4)		(4)		(4)
Nov.	0.26	0.32	0.16	0.20	0.10	(0.07)	0.11	(0.09)	0.15	(0.10)
Dec.	0.09	0.11	0.13	0.17	0.08	(0.07)	0.10	(0.07)	0.11	(0.07)
Jan.	0.12	0.10	0.14	0.17	0.04	(0.05)	0.06	(0.08)	0.09	(0.03)
Feb.	0.07	0.09	0.12	0.15	0.09	(0.08)	0.08	(0.08)	0.11	(0.13)
Mar.	0.09	0.07	0.07	0.08	0.07	(0.10)	0.07	(0.08)	0.10	(0.09)
April	0.10	0.09	0.10	0.16	0.07	(0.08)	0.08	(0.09)	0.11	(0.11)
May	0.08	0.09	0.07	0.12	0.07	(0.07)	0.07	(0.06)	0.16	(0.03)
Means										
Nov.- May incl.	0.116	0.124	0.113	0.150	0.074	(0.074)	0.081	(0.079)	0.119	(0.094)
Feb.–May incl.	0.085	0.085	0.090	0.128	0.075		0.075		0.115	

1. Paddocks 7A and 7B grazed as a single unit from 2 April 1952.
2. Grazing discontinued after 22 April 1952.
3. Assessed from results shown in Table 2 and Fig. 2.
4. Samples from frames.

relatively constant through to May. This is reflected in the appreciably higher means for November to May as compared with those for February to May. In contrast, values remained relatively constant from November to May during the 1952–53 season, and for this season there are no appreciable differences between the means found for November to May and those for February to May.

Effects of Grazing Treatments. For the 1951–52 season there is no significant difference between results found for samples from 7B and those from 7A. The slightly higher mean for November to May for 7A results from the higher figure for the November sample from this paddock; means for February to May are the same. On the other hand, results for samples taken from the lightly grazed paddock 11 in the 1951–52 season are in all cases lower than the corresponding results for samples from the heavily grazed paddock 10. In the following season, when grazing treatments were reversed, results for samples from paddock 11 are in all cases higher than corresponding figures for samples from paddock 10.

A comparison of results for samples from paddocks with those for samples from corresponding frames (1952–53 season) shows that under light grazing conditions (paddocks 7 and 10) there is fair agreement between contemporaneous samples. Mean values for November to May are virtually identical. By contrast, under heavy grazing conditions (paddock 11), figures for samples from the paddock are in most cases higher than corresponding figures for samples from the frames, and result in a higher mean.

Cobalt Contents of Livers

Table 2 shows the cobalt contents of livers from experimental lambs during the two seasons.

TABLE 2. COBALT CONTENT (p.p.m. D.M.) OF LIVERS

Paddock No.	Grazing Treatments			
	1951–52		1952–53	
	Light	Heavy	Light	Heavy
7A	0.03	—	0.04*	—
7B	—	0.07, 0.06, 0.02		
10	—	0.16	0.05, 0.06	—
11	0.03, 0.05	—	—	0.13, 0.13

* Mean of 4 samples (range 0.02–0.06 p.p.m.)

For paddock 7, results are indicative of cobalt deficiency under both light and heavy grazing, and during both seasons.

For paddock 10, results suggest a sufficiency of cobalt under heavy grazing during the 1951–52 season, but a deficiency of cobalt under light grazing during the following season. Similarly for paddock 11 cobalt deficiency is indicated under light grazing in the 1951 52 season, but a sufficiency is indicated under heavy grazing during the 1952–53 season.

Weight Changes and Responses to Cobalt

Fig. 2 shows mean-weight changes and cobalt and vitamin B_{12} responses of lambs in experimental paddocks during each of the two seasons.

1951–52 season. Comparing performances in paddocks 7A and 7B, by mid November the mean weight gain of lambs in the heavily grazed paddock (7A) was 3.3 lb less than that of lambs in the lightly grazed paddock (7B). By mid January, however, the situation had reversed and the mean gain of lambs in 7A was 4.3 lb. greater than the corresponding value for lambs in 7B. After 4 February, mean weights of lambs declined in both 7A and 7B. Between the beginning of the trial on 12 October 1951 and 2 April 1952, when grazing treatments finished, lambs in paddock 7A gained 7.7 lb. more than lambs in paddock 7B. This difference does not reach significance at $P = 0.05$. But between 4 February and 2 April 1952 the mean weight loss of lambs in 7B was 14.2 lb. whereas that for lambs in 7A was 9.3 lb. The difference of 4.9 lb in favour of lambs on heavily grazed pasture is statistically significant ($P < 0.05$).

Between 2 April and 4 June, when lambs from paddocks 7A and 7B were run together over the whole of paddock 7 and regrouped, groups given cobalt orally and vitamin B_{12} by injection responded immediately and significantly to treatments (Anderson and Andrews 1952). These results supplement those given in Table 2, and show that the unthriftiness of lambs in both paddocks 7A and 7B was due to cobalt-deficiency disease.

Comparing results for lambs in paddocks 10 and 11, by 4 February 1952 those on the heavily grazed pasture had gained on an average 8.9 lb less than those on the lightly grazed pasture. Thereafter, the situation changed abruptly. All lambs on heavily grazed pasture continued to gain weight and between 4 February and 24 April there was a further mean increase of 11.8 lb. During the same period all lambs on lightly grazed pastures declined in weight, the mean loss being 6.6 lb. Over the whole experimental period lambs on heavily grazed pasture gained on an average 9.5 lb more than those on the lightly grazed pasture. Although it was not possible to conduct cobalt-response trials in paddocks 11 and 10 during this season, the patterns of mean weight changes together with results from liver analyses indicate cobalt deficiency among lambs on lightly grazed pasture and no deficiency among those on heavily grazed pasture.

1952–53 Season. In paddock 7, lambs gained weight until 30 December. 1952. Thereafter, they deteriorated and between 30 December 1952 and 26 February 1953 the mean weight loss was 7.1 lb. Between 26 February and 10 June, groups given cobalt orally, and oral and injected doses of vitamin B_{12}, responded significantly to treatments (Andrews and

Anderson 1954). These results supplement those given for paddock 7 in Table 2 and show that, as in the previous season, lambs in paddock 7 were cobalt-deficient.

Comparing performances of lambs in paddocks 10 and 11, for the first 4 months lambs on the heavily grazed pasture (now paddock 11) gained weight at a slower rate than those on the lightly grazed pasture (now paddock 10). After 26 February, however, the situation changed in a manner closely similar to that obtaining in the previous year. All lambs on heavily grazed pasture continued to gain weight, and by 22 April had increased their mean gain by 4.3 lb. During the same period, except for one lamb that maintained its weight, all others on the lightly grazed pasture lost weight, the mean loss being 5.5 lb. Over the whole experimental period, lambs on the heavily grazed pasture gained on an average 6.5 lb more than those on the lightly grazed pasture. Between 22 April and 21 May, cobalt-response trials showed that there was virtually no difference between the control sub-group (mean gain 0.1 lb) and the cobalt-dosed sub-group (mean gain 0.5 lb) on heavily grazed pasture. But on lightly grazed pasture mean weight changes were −1.0 lb for the control sub-group, and +6.6 lb for the cobalt-dosed sub-group, the 7.6 lb difference being statistically significant ($P < 0.05$). The findings confirm those indicated in Table 2 and show that after reversal of grazing treatments cobalt-deficiency disease was again associated with light grazing, but not with heavy grazing.

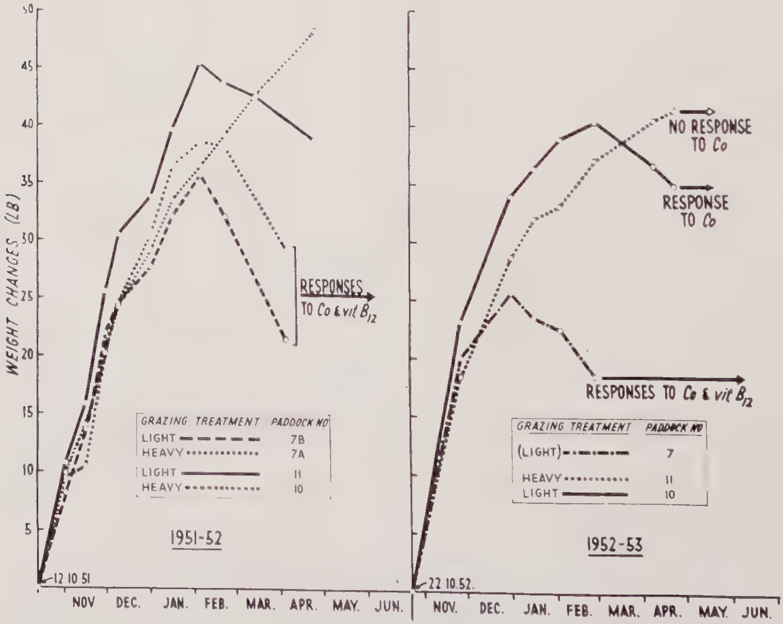


Fig. 2.—Weight changes and responses of experimental lambs to cobalt and vitamin B_{12}.

Relationship Between Cobalt Status of Lambs and Pasture-associated Cobalt

Results given in Fig. 2 and Table 1 show that from February 1952 weight losses due to cobalt deficiency among 1951–52 season's lambs (paddocks 7A, 7B, and 11) were associated with pasture samples containing on an average about 0.09 p.p.m. cobalt. In the following season, weight losses from February 1953 (paddocks 7 and 10) were associated with pasture samples containing on an average about 0.08 p.p.m.

From February of each season, samples from cobalt-sufficient pastures contained an average of about 0.13 p.p.m. in 1952 (paddock 10), and about 0.12 p.p.m. in the following year (paddock 11).

DISCUSSION

Factors Other Than Grazing Treatments That May Influence the Degree of Severity of Cobalt-deficiency Disease

Fig. 2 shows that, during the 1951–52 season, lamb unthriftiness in paddocks 7A and 7B was more marked than in the lightly grazed paddock 11. In the following season, obvious unthriftiness developed earlier and was more severe among lambs in paddock 7 than among those in paddock 10, when both these paddocks were lightly grazed. It follows that a factor (or factors) other than grazing intensity was responsible for the greater lack of thrift shown by lambs in paddock 7. While means for February to May 1951–52, and November to May 1952–53 (Table 1), are consistent with the idea that the greater lack of thrift of lambs in paddock 7 resulted from a lower cobalt intake, the evidence is at best indefinite. But this could be ascribed, at least in part, to the inability of results from samples taken at relatively long intervals to reflect with sufficient sensitivity small and variable differences in the amounts of cobalt continuously ingested from pastures. In the absence of an alternative explanation, it is reasonable to suppose that lambs in paddock 7 were more unthrifty because their food provided them with less cobalt. So far as is known, the fact that cultivation and cropping had relatively recently been carried out in paddock 7, but not in paddocks 10 and 11, represents the only major difference between the former and the latter two paddocks. Hence the possibility that cultivation and cropping can influence the amount of cobalt subsequently taken up by pasture plants seems to warrant further investigation.

Effects of Grazing Treatments on Pasture-associated Cobalt and on Cobalt-deficiency Disease

Comparison of results given in Table 1 for pasture samples from paddocks 7A and 7B during the 1951–52 season do not show that heavy grazing in the former paddock was accompanied by increases in the amounts of pasture-associated cobalt. On the other hand results for paddocks 10 and 11 show that during both seasons cobalt figures for samples from heavily grazed pastures were in all cases higher than corresponding figures for samples from lightly grazed pastures. Also a comparison of figures for samples from paddocks with those from frames suggests that heavy grazing, but not light grazing, increased the average amount of pasture-associated cobalt. The lack of appreciable difference between the mean for November to May for samples from paddock 7A and that for samples from paddock 7B could be explained by failure to keep pastures sufficiently short in the former paddock. If this explanation is accepted, results given in Table 1 show that if pastures are grazed sufficiently short the amounts of cobalt associated with them are increased.

Although in the 1951–52 season lambs in both paddocks 7B and 7A developed cobalt-deficiency disease, by the end of the trial unthriftiness was somewhat less severe among those in the heavily grazed paddock. That this difference was due to the different treatments is supported by results from the replicated block (paddocks 11 and 10) which also showed that heavy grazing was ultimately associated with better thrift. In the replicated block, lambs in the lightly grazed paddock 11 grew well until February, but thereafter weight losses followed a pattern similar to those of cobalt-deficient lambs in paddocks 7A and 7B. This, in conjunction with low-cobalt livers, indicated cobalt deficiency in lambs in paddock 11. Presumably owing to limitations imposed by restricted feeding, lambs in the heavily grazed paddock 10 grew at a slower rate than did lambs in paddock 11 until February, but showed no evidence of cobalt deficiency as judged by subsequent weight gains and a liver normal in cobalt content. The 1952–53 findings confirm those for paddocks 10 and 11 in the previous season. Reversal of grazing treatments gave a closely similar growth pattern. Light grazing again resulted in unthriftiness due to cobalt deficiency; heavy grazing was again associated with initially slower growth but no cobalt deficiency.

Two explanations could account for the failure of heavy grazing to prevent cobalt deficiency completely, in lambs in paddock 7A during the 1951–52 season. Firstly, as previously discussed, the pasture may not have been grazed sufficiently short. The visibly greater length of pasture in paddock 7A as compared with that in paddock 10, and the relatively inappreciable differences between mean cobalt figures for November to May for pastures in paddocks 7A and 7B, and between growth rates of lambs in these paddocks during the first two months, support this possibility. Secondly, and perhaps more importantly, the degree of deficiency among lambs in the 7A and 7B block may have been too severe to be completely preventable by heavy grazing.

Overall, we interpret the results from pasture and liver analyses, together with those from the lamb trials, as showing that light grazing favours cobalt-deficiency disease; heavy grazing tends to prevent it. Prevention can be partial or complete and may depend upon the degree of deficiency to which lambs are exposed.

Present results confirm experimentally the observation made by Dixon (1936) that "keeping the grass short . . . is distinctly beneficial to the sheep". They do not, however, explain the higher pasture-cobalt figures and decreased susceptibility of lambs to cobalt deficiency on short pastures. But it is to be expected that short, well-trampled pastures will be more prone to soil contamination than long less-trampled pastures. Since soil invariably contains much more cobalt than does pasture (McNaught and Paul 1939) contamination would result in increased amounts of pasture-associated cobalt. The cobalt content of soil samples from paddocks 10 and 11 was about 3 p.p.m. (unpublished data) so that only one or two per cent of soil as a pasture contaminant would be needed to account for the increased amounts of cobalt available from heavily grazed pastures. Although it is probable that pasture cobalt is more effectively utilised than soil cobalt, other work (Dixon 1936; Askew and Dixon 1936) has shown that oral doses of soil will prevent cobalt deficiency disease in sheep. From the evidence, we consider that soil contamination of short-grazed pastures offers the most probable explanation of their higher cobalt figures and of the protection against cobalt-deficiency disease afforded by heavy grazing.

The Minimal Level of Pasture-associated Cobalt Required by Lambs

For the purposes of this discussion the "minimal level" is defined as the lowest average concentration of pasture-associated cobalt that will continuously satisfy the cobalt requirement of grazing sheep.

A number of investigators have made estimates of the minimal level for sheep. McNaught's (1938) estimate was 0.07 p.p.m. This was based on many analyses of pasture samples from cobalt-deficient and apparently normal areas in the North Island of New Zealand. No attempt was made to differentiate between a requirement for mature sheep and for lambs. But in a later publication (McNaught and Paul 1939) it was stated that "pastures containing less than about 0.08 p.p.m. . . . must be regarded in normal circumstances as deficient for sheep, especially lambs". Marston's (1952) estimate, presumably for sheep in general was 0.08 to 0.10 p.p.m. Stewart (1951) states that the "cobalt content of Scottish pastures on which lambs pine ranges from 0.10 to 0.03 p.p.m.", but from examination of the original data on which this statement was based (Stewart, Mitchell and Stewart 1941) it is not evident whether the figure of 0.10 is regarded as the highest of a range of values, or as an average for samples taken over a period from pastures known to be cobalt-deficient for lambs.

It seems very doubtful whether any estimate of a minimal level for sheep under a given set of conditions will have general applicability. Since lambs are more sensitive than mature sheep to cobalt deficiency, they probably require a higher concentration of cobalt in their feed. Rapidly growing lambs may need relatively more cobalt than those growing at a slower rate. Again, it is by no means certain that the animal can utilise cobalt equally well from different feeds, or from "clean" and soil-contaminated pasture. Under the particular conditions of our experiments, results show that samples from short and probably soil-contaminated pastures providing adequate amounts of cobalt contained on an average about 0.12 p.p.m. or more. Samples from long and presumably relatively "clean" pastures on which lambs lost weight contained on an average about 0.09 p.p.m. or less. It seems likely that milder cobalt-deficiency disease manifested by stationary weights or sub-optimal gains could be associated with pastures containing on an average as much as 0.10 p.p.m. Hence, assuming that the utilisation of cobalt from "clean" pastures is not greatly different from that of cobalt from soil-contaminated pastures, we consider that within a range of conditions similar to those of our trials a figure of 0.11 p.p.m. approximates the minimal level of pasture-associated cobalt needed by lambs.

Cobalt Deficiency and Soil Type

Dixon (1936) described early investigations in Southland into a wasting disease of lambs, subsequently found to be due to a deficiency of cobalt. At that time the ailment was known as "Morton Mains disease" because of its prevalence in that district, but Dixon states that it could occur at Edendale, Waipango, and Otara. Affected soils were described as being derived from loess, although not all loess soils were necessarily associated with the disease. As a result of a more recent and intensive survey of Southland soils, that type associated with cobalt-deficiency disease in the Morton Mains area is now provisionally described by the New Zealand Soil Bureau (Mr. E. J. B. Cutler, pers. comm.) as follows. *"Waikiwi soils* (*Established*). Waikiwi soils are strongly leached yellow-brown earths formed on loess of greywacke origin on a land surface dating from late Pleistocene times. The vegetation at the time of European settlement was mainly red tussock (*Danthonia rigida*) but in earlier times the vegetation was broadleaf-podocarp forest. Area, approx. 76,000 acres."

The present work was carried out on a different soil type not hitherto recognised as being associated with cobalt-deficiency disease in Southland and which, as previously indicated, is provisionally classified as Otapiri silt loam. This soil type lies within the Southland Plain, east of the Oreti River. It extends from about 5 miles north of Makarewa through Winton and Otapiri to about 4 miles south of Dipton. Mr. Cutler describes it as follows. *"Otapiri soils, Otapiri silt loam* (*Established*). Otapiri soils are gley soils related to soils transitional between yellow-grey earths and yellow-brown earths. They are formed on alluvium or possibly loess of greywacke origin on a land surface of late Pleistocene age. The vegetation at the time of European settlement was mainly red tussock with patches of flax in the wetter hollows. The soil is moderately to strongly leached and has impeded drainage It has been extensively drained but is still wet in winter. Because it lies in a region of low summer rainfall the upper horizons may, however, dry out in dry years. Area, approx. 23,000 acres". Livers from some farms within the area have shown a low or borderline cobalt content, and some farmers claim a good response of lambs to cobalt. This, in conjunction with present findings, shows that Otapiri silt loam can be added to those soil types in New Zealand described (Andrews 1956) as marginally cobalt-deficient.

ACKNOWLEDGEMENTS

We are indebted to Mr. E. J. B. Cutler of the New Zealand Soil Bureau for soil data, to various members of the staff of the Department of Agriculture, Invercargill, for assistance with the field trials, and to the Winton Experimental Farm Committee, who provided facilities for the work.

REFERENCES

ANDERSON, J. P.; ANDREWS, E. D. 1952: Response to Vitamin B_{12} of Grazing Cobalt-deficient Lambs. *Nature, Lond. 170:* 807.

ANDREWS, E. D. 1956: Cobalt Deficiency. *N.Z. J. Agric. 92:* 239-44.

ANDREWS, E. D., ANDERSON, J. P. 1954: Responses of Cobalt-deficient Lambs to Cobalt and to Vitamin B_{12}. *N.Z. J. Sci. Tech. A35:* 483-8.

ASKEW, H. O.; DIXON, J. K. 1936: The Importance of Cobalt in the Treatment of Certain Stock Ailments in the South Island, New Zealand. *N.Z. J. Sci. Tech. 18:* 73-92.

DIXON, J. K. 1936: Investigations on a Sheep Ailment in Southland. *N.Z. J. Sci. Tech. 17:* 600-19.

MARSTON, H. R. 1952: Cobalt, Copper, and Molybdenum in the Nutrition of Animals and Plants. *Physiol. Rev. 32:* 66-121.

McNAUGHT, K. J. 1938: The Cobalt Content of North Island Pastures. *N.Z. J. Sci. Tech. A20:* 14-30.

——————1949: Cobalt, Copper and Iron in the Liver in Relation to Cobalt Deficiency Ailment. *N.Z. J. Sci. Tech. A30:* 26-43.

McNAUGHT, K. J.; PAUL, G. W. 1939: Seasonal Variation in Cobalt Content of North Island Pastures. *N.Z. J. Sci. Tech. B21:* 95-101.

STEWART, J. 1951: Induced Cobalt Deficiency in Lambs. *Brit. J. Nutrit. 5:* 320-26.

STEWART, J.; MITCHELL, R. L.; STEWART, A. B. 1941: *Emp. J. exp. Agric. 9:* 145-52.

BLOAT IN CATTLE

VII. THE MEASUREMENT OF FOAMING PROPERTIES OF SURFACE-ACTIVE COMPOUNDS

By J. L. MANGAN, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publication, 4 November 1957)

Summary

An apparatus is described for the estimation of the expansion, dynamic stability and strength of foams formed by surface active compounds. Three solutions may be examined simultaneously.

Foam strength is the most useful measurement and has been used to demonstrate that the properties of lucerne saponin foams are pH dependent whereas yucca saponin foams are not.

The possible significance of these measurements in the problem of bloat is briefly discussed.

INTRODUCTION

Previous papers in this series (Johns 1954; Reid & Johns 1957) have established that bloat in cattle feeding on legumes under New Zealand conditions is caused by foaming of the rumen ingesta, fermentation gases being retained in the form of a stable foam which cannot be eliminated by the normal eructation mechanism. The work of Clark & Weiss (1952) in South Africa, of Nichols (1954), Hungate *et al.* (1955) and Barrentine (unpub.) in U.S.A., Ferguson and Terry (1955) in England and Geddes (unpub.) in Australia, indicates that "foamy" bloat occurs in other countries.

Although bloat is a disorder involving numerous plant and animal factors, the identification of the active foaming agents is of major importance in the elucidation of the cause of the ailment. Attempts have been made by previous workers to correlate foaming of legume extracts and rumen liquor with the incidence of bloat. Thus Ferguson and Terry (1955) considered that the foaminess of lucerne juice during preparation gave a fair indication of its bloating potential. Weiss (1953), however, could find no significant variation of foaming capacity of lucerne juices, as judged by simple shaking, over a considerable growth period. Jacobson and Lindahl (1955) measured "ingesta volume increase" by incubating freshly withdrawn rumen liquor at 39°C. After reading the subsequent increase in volume, the sample was stirred 12 times and the "stable ingesta volume increase" was read. Lindahl and Davis (1955) improved this method by adding a standard amount of glucose before incubation. Hungate *et al.* (1955) found that during incubation of rumen liquor in Warburg vessels there was some correlation between foam production and bloat.

None of these authors considered the type of foam or the physico-chemical requirements for stable foam formation or studied the foaming properties of any of the surface-active agents known to be ingested into the rumen.

Plateau (1868, 1870, 1873) in his classical studies has shown that surface-active compounds may be one of two types: (a) Surface-viscous compounds, such as proteins and saponins, which yield highly stable foams; their solutions show relatively little lowering of surface tension and their foam stability is correlated with their high surface viscosity. (b) Low-surface-tension compounds, such as the soaps, which form less rigid foams, and have low surface viscosity. The foaming properties of their solutions is related to their low surface tension.

A number of properties of foams may be measured, e.g. Clark (1947) in his studies on fire-fighting foams describes methods for measuring the expansion, viscosity, static and dynamic stability, and bubble size of foams. In addition, static and dynamic interfacial tension and interfacial viscosity measurements on the foaming solution were considered to be of importance. It is difficult to decide, however, what properties of foams should be measured to be of significance in bloat studies. Nichols *et al.* (1956, 1957) and Blake *et al.* (1957) have measured surface tension changes in rumen liquor, but Reid and Johns (1957) have pointed out that the foaming system in the rumen is of the surface-viscous type. Surface-tension measurements would then appear not to be relevant to rumen foams. Johns (1954) showed that foam obtained by shaking rumen liquor from clover-fed sheep had a much greater stability than that from grass-fed sheep and considers that the stability of the foam is the most important property and would determine whether or not the gas would be released to form a pocket of free gas in the dorsal sac of the rumen.

The present paper describes apparatus for the measurement of foam expansion, dynamic foam stability, and foam strength, properties which are relevant to surface-viscous foams.

FOAM-MEASURING APPARATUS

The dynamic foam meter of Clark (1947), derived from Foulk and Miller (1931), has been modified to provide for the measurement of foam strength and to allow three estimations to be carried out simultaneously. The apparatus (Fig. 1) consists essentially of a Perspex box (A) in which are mounted three glass tubes (B) 35 mm internal diameter, 64 cm in height, and carefully selected for uniformity of bore. The Perspex box is provided with inlet and outlet tubes for the circulation of water from the constant temperature (37°C) bath (C), and is calibrated from 0 to 45 cm by horizontal lines scored into the front and back surfaces of the box to avoid parallax errors. The bottom ends of the glass tubes are closed with rubber bungs carrying a grade 2 sintered-glass sparger (D) 30 mm diameter, and an inlet tube for the solution to be foamed. The apparatus is mounted so that the glass tubes can be kept firmly in a perpendicular position. The gas to be used is contained in a 25-litre bottle (E) kept under a constant head of 1.3 metres of water pressure by the overflow device (F). The gas is saturated with water vapour and prevents undue evaporation

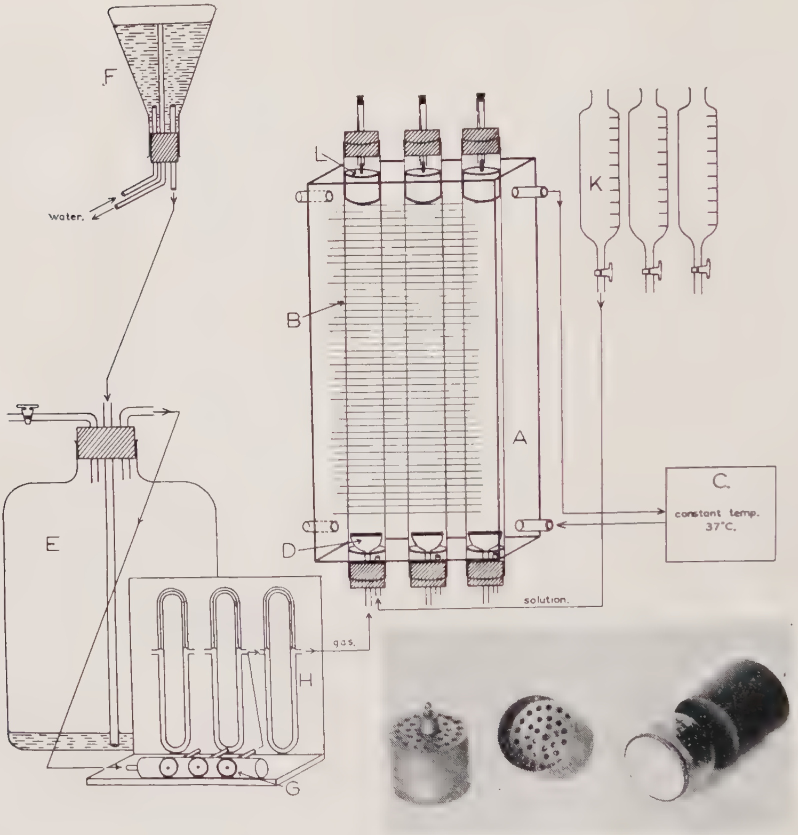


Fig 1.—Foam-measuring apparatus (details in text); inset, sintered glass sparger and weights for measurement of foam strength.

of the solution during foaming. The gas flow to the spargers is controlled by small needle valves (G) and adjusted to 25 cc/min on the flowmeters (H). The level of the solution under examination is maintained at the 0 cm graduation by additions from the graduated reservoirs (K). The depth of solution over the spargers is 45 mm so that the gas bubbles have ample time to come to equilibrium before entering the foam zone.

Dynamic Foam Stability

In the apparatus described, gas is passed through the spargers at a standard pressure and rate of flow. The solution to be examined is then run in to the 0 cm mark, the timer started, and the gas flow re-adjusted. As liquid is used in foam formation, the liquid level is maintained at 0 by additions from the reservoirs. The foam height is plotted against time. If the foam produced has perfect stability there will be a straight line relationship, but if the foam is partially breaking down curved plots are obtained as shown in Fig. 2. A standard time

(15 min) is taken and foam stability is expressed as a percentage of the height obtained with perfect stability.

Dynamic foam stabilities can be calculated from foam gas volumes rather than foam heights and are then independent of the wetness of the foams and hence a little more accurate (Clark 1947). Foam heights are converted to foam volumes using a calibration curve, and, by subtracting the volume of liquid added from the reservoir during foaming, the foam gas volume is obtained.

Limiting Expansion

Limiting expansion is expressed as the maximum number of volumes of foam obtainable from one volume of solution. Provided this does not exceed the capacity of the tubes, it is recorded when dynamic stability is measured.

Foam Strength

Foam strength is measured by the rate of fall of a perforated brass weight through the foams produced under reproducible conditions in the apparatus described above. The weights (L), of mass 120 g, are in the form of a piston machined to fit the glass tubes with 1-mm clearance and drilled with 35 holes 2-mm diameter as shown in Fig. 1 inset. For the measurement, the weight, suspended by a nylon thread, is lowered gently onto the foam column until some foam or liquid oozes through the top of the perforations. The position of the weight is noted, it is then released and the drop of the weight plotted against time.

Foam strength is inversely proportional to the rate of drop and is expressed as 100/slope of plotted line. The conditions of the measurement are quite arbitary, but foam strength is a function of the shearing strength of the foam and gives an easily measured index of the rigidity or resistance to distortion or breaking of the foam. Under these conditions saponin or protein foams have drop times ranging from 2 seconds to an estimated 10 hours for 45 cm of foam, indicating a wide range of orders of foam strength.

In constructing this apparatus containing three columns, some difficulty occurs in obtaining uniformity of the component parts. The brass weights can be turned and drilled accurately and small differences in mass may be corrected by adding rings to the holding pins on top of the weights. Selection of the glass tubing and spargers, however, is by trial and error. With 0.5% yucca saponin as test solution, both the rate of expansion of the foam and the rate of fall of the weights through the foams produced are used as tests of uniformity when selecting the glass tubes and spargers.

RESULTS

Foam Expansion and Dynamic Foam Stability

The measurement of dynamic foam stability is illustrated in Fig. 2a with sodium oleate solutions of different concentrations. The series of

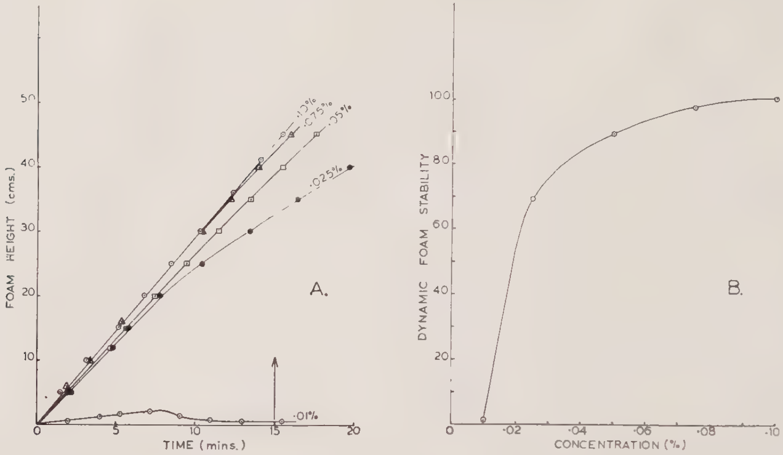


Fig. 2.—Dynamic foam stability of sodium oleate solutions.
(A) Rate of foam expansion at different concentrations.
(B) Dynamic foam stability as a function of concentration.

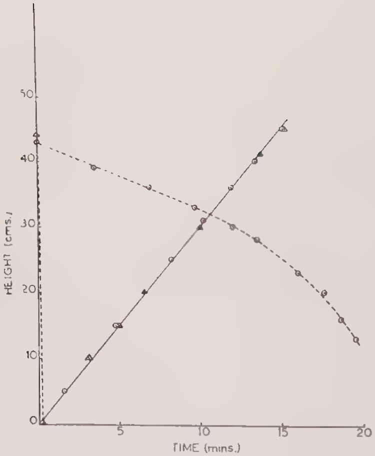


Fig. 3

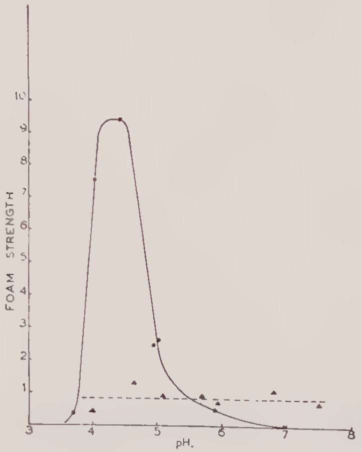


Fig. 4

Fig. 3.—Comparison of dynamic foam stability and foam strength measurements of sodium oleate (0.1%) and salivary mucoprotein (5 mg N/100 ml) solutions.
Expansion of sodium oleate ——△—— and salivary mucoprotein ——⊙—— ; foam strength of sodium oleate - - - -△- - - and salivary mucoprotein - - - -⊙- - - - -

Fig. 4.—Effect of pH on foam strength of saponin foams.
——●—— Crystalline lucerne saponin, 0.1% in acetate buffers.
- - - -▲- - - - Commercial yucca saponin, 0.2% in acetate buffers.

curves shows a range in foam stabilities, the 0.1% solution giving a straight line which represents "perfect" foam stability. Taking 15 minutes as the standard time, the foam heights expressed as a percentage of that of the 0.1% solution represent the foam stabilities of the other solutions. In Fig. 2b, foam stability is plotted against concentration of sodium oleate. The dynamic foam stability rises very rapidly between 0.01% and 0.025% sodium oleate, followed by a gradual increase until at 0.10% sodium oleate "perfect" foam stability is attained.

In a similar way foam expansion increases from a small value of 0.3 at 0.01% concentration to a value greater than 6.6, the maximum measurable in the apparatus, at a concentration of 0.025%.

Foam expansion and dynamic foam stability are useful measurements with pure solutions of surface-active agents but restricted to low concentrations. With rumen liquor it is only useful where foaming is restricted by the presence of antifoaming agents, as the foam stability of the active agents is in general of a higher order of magnitude.

Dynamic Foam Stability and Foam Strength

It is possible to have foams of similar appearance and identical foam stability and yet giving very different foam-strength values. An example of this is given in Fig. 3 where the foaming properties of sodium oleate (0.10%) and bovine salivary mucoprotein (5 mg N/100 ml) are compared. The appearance of the foams was almost the same, both being good fine-bubble foams. The dynamic foam stabilities were identical, but, as shown by the rate of fall of the weights, the foam strengths were very different, the sodium oleate foam possessing very little resistance to mechanical stress. It has been observed in numerous cases that where there is a visual difference between foams possessing identical foam stability, this difference is also reflected in differing foam strengths.

The curve obtained for salivary mucoprotein demonstrates that foam-strength measurements are a check on the uniformity of the foam. The top portion of this foam is obviously more resistant to mechanical stress than the lower part. At low concentrations of foaming compound this behaviour is to be expected since the surface-active agent concentrates in the foam and the solution below is gradually depleted. The foam strength can be expressed in two ways, either as the maximum foam strength considering only the straight-line part of the curve, or as the average foam strength for the complete drop of the weight.

The effect of pH on the foaming properties of lucerne saponin further demonstrates the usefulness of foam-strength measurements. The saponin was crystalline material prepared by the cholesteride method (Walter *et al.* 1954) and kindly supplied by Dr. C. R. Thompson, Western Regional Laboratories, California. Foam strength (Fig. 4) showed a well defined maximum at pH 4.5. Dynamic-foam-stability measurements showed only a poorly defined maximum in the same pH range, and illustrates again the superiority of foam-strength measurements.

Lucerne saponin has a triterpenoid structure containing free carboxyl groups (Walter *et al.* 1954) and it would appear that the state of ionisation of these groups is concerned in the surface activity of the compound. It seems possible that a condition akin to that of proteins, where maximum foaminess occurs at the isoelectric point, is also necessary for high foam strength for this type of saponin. An interesting comparison can be made with yucca saponin which has a steroidal structure devoid of ionising groups. Changes in pH over the range 4.0 to 7.5 were without effect on foam strength (Fig. 4).

DISCUSSION

Measurements of dynamic foam stability, without further description of the properties of the foam produced, cannot be considered to give useful information. Two foams of quite different appearance, particularly bubble size, can give almost the same figure for dynamic foam stability. Visible breakdown in the body of the foam, with small bubbles coalescing to form larger bubbles, and thus giving rise to a coarser and weaker foam, quite often occurs. But the above measurements, depending on the height or volume of foam produced, do not record this effect unless bubbles on the top surface of the foam are breaking completely, thus giving a reduction in foam volume. With foams in which the concentration of foaming agent is highest in the top layers, this breakdown is unlikely. In contrast, foam-strength measurements do record differences not detected in other foam-stability determinations. This is illustrated in Fig. 4 in the case of lucerne saponin.

In relating foam-strength measurements to bloat, it should be noted that the rumen ingesta, consisting of a mass of plant material interspaced with fluid from which the foam arises, is in a more or less continuous state of movement. It would be expected that for a foam to persist in the rumen it must have considerable resistance to mechanical stress. The foam bubbles will be in a constant state of movement one against the other, and in addition will be squeezed between plant material by the churning movement of the ingesta and by the changes in pressure during rumen-reticular movements. It is reasonable to assume that foam stability in the rumen is directly dependent on the foam being able to withstand this treatment. Measurement of foam strength as described above, although arbitrary, is considered to give a measure of the ability of a foam to resist mechanical rupture, and in work in this laboratory the greatest importance has been attached to this property of foams. The results of the use of this technique in the study of bloat will be presented in a further paper.

ACKNOWLEDGEMENTS

My thanks are due to Miss M. E. Brown and Mr. J. G. Fraser for their technical assistance.

REFERENCES

BLAKE, J. T.; ALLEN, R. S.; JACOBSON, N. L. 1957: *J. Anim. Sci. 16*: 190.

CLARK, N. O. 1947: Mechanically Produced Foam for Combating Petrol Fires. H.M. Stationery Office, London.

CLARK, R.; WEISS, K. E. 1952: *J. S. Afr. vet. med. Assn. 23:* 163.

FOULK, C. W.; MILLER, J. N. 1931: *Industr. Engng Chem. 23:* 1283.

FERGUSON, W. S.; TERRY, R. A. 1955: *J. agric. Sci. 46:* 257.

HUNGATE, R. E.; FLETCHER, D. W.; DOUGHERTY, R. W.; BARRENTINE, B. F. 1955: *App. Microbiol. 3:* 161.

JACOBSON, D. R.; LINDAHL, I. L. 1955: *Misc. Publ. Univ. Md. 238:* 9.

JOHNS, A. T. 1954: *N.Z. J. Sci. Tech. A36:* 289.

LINDAHL, I. L.; DAVIS, R. E. 1955: *Proc. 3rd Conf. on Rumen Function,* Chicago.

NICHOLS, R. E. 1945: *Vet. Med. 49:* 91.

NICHOLS, R. E.; DILLION, R. D.; PENN, K.; BRYANT, J.; SCHREIBER, J. 1956: *Vet. Med. 51:* 389.

NICHOLS, R. E.; PENN, K.; SCHREIBER, J. 1957: *Vet. Med. 52:* 37.

PLATEAU, J. 1868: *Mém. Acad. Belg. 37:* 1.

——————1870: *Pogg. Ann. 141:* 44.

——————1873: *Stat. des Liquides,* Paris.

REID, C. S. W.; JOHNS, A. T. 1957: *N.Z. J. Sci. Tech. A38:* 908.

WALTER, E. D.; VAN ATTA, G. R.; THOMPSON, C. R.;; MACLAY, W. D. 1954: *J. Amer. chem. Soc. 76:* 2271.

WEISS, K. E. 1953: *Onderstepoort J. vet. Res. 26:* 251.

POTASSIUM DEFICIENCY IN PASTURES

I. POTASSIUM CONTENT OF LEGUMES AND GRASSES

By K. J. McNaught, Rukuhia Soil Research Station, Hamilton

(Received for publication, 20 June 1957)

Summary

Legumes and grasses were analysed from two series of trials (a) pure species trials (b) mixed pastures from which legumes and grasses were dissected out for separate analysis.

In the pasture trials on Horotiu sandy loam under cow grazing, yield responses to potassium fertilisers commenced in September, reached a maximum in summer (November to January), declined in autumn and were at a minimum in winter. The appearance of deficiency symptoms in white and red clovers corresponded to this pattern of growth responses which followed the seasonal growth cycle. Symptoms were not detected in winter.

On deficient soils, uptake responses to potassium fertilisers were appreciable over the whole range from severe deficiency to adequacy, but were small or not detectable on soils with high available potassium.

Large differences in analytical levels were found in different tissues of plants well supplied with potassium. Differences were much smaller in potassium-deficient plants.

Seasonal decline in the potassium content of pasture species in autumn may have been due mainly to differences in maturity of tissues, associated with longer intervals between grazings, and to "carbohydrate dilution".

Clover leaves (leaflets plus petioles), with deficiency symptoms, showed potassium levels below 0.7%.

The critical level, or minimum level for near-maximum growth, was approximately 1.8% K for whole leaves (immature plus mature) of white and red clover at grazing height. The value for ryegrass was approximately 1.6% K and similar levels were indicated for cocksfoot and Yorkshire fog. Sweet vernal and especially paspalum appear to have much lower needs. The gap between the maximum level in clover leaves showing symptoms and the apparent critical level in relation to yield may be partly due to (a) differences in maturity and type of tissues analysed, (b) soil heterogeneity, (c) other limiting growth factors.

In pastures on potassium-deficient soils, grasses are more efficient at securing their potassium needs than associated clovers. When ryegrass-dominant pastures are analysed for the purpose of predicting responses of the clover component to potassium fertiliser, higher reference levels may be required than for pure clover stands.

INTRODUCTION

To obtain suitable plant material for evaluating plant analysis as a research aid and as a technique for deficiency diagnosis, selected pure pasture species were grown as annual crops in experimental plots on two different soil types, potassium being one of several nutrients under simultaneous study. In addition, samples of mixed pasture were collected from various fertiliser trials with permanent pasture, and the clovers and grasses dissected out for separate analysis. Both total and

N.Z. J. agric. Res. 1: 148–181

readily extractable nutrients were determined on many samples. The present paper summarises the results of the potassium investigations and is restricted to consideration of total potassium concentrations. Interrelationships between potassium and other cations will be discussed in a separate paper.

EXPERIMENTAL

Trials with Pure Species

Experiment 1. Hamilton clay loam, 1946-47. In November 1946, a randomised block experiment was laid down, at the Rukuhia Soil Research Station, on Hamilton silty clay loam, a semi-mature brown granular loam from andesitic ash, testing as follows (0–6 in. depth): pH 5.7; cation-exchange capacity 22 m.e.%; exchangeable cations (m.e.%): calcium 9.6, magnesium 1.2, potassium 1.2; available phosphorus (Truog) 4.6 mg % P_2O_5; fixation, medium. There were six replicates, three limed, three unlimed, of the following treatments, Control, PK, PN, KN, PKN, PKNMg plus trace elements, compost. The per-acre rates were as follows: P — superphosphate 10 cwt., K — potassium sulphate 2 cwt., N — ammonium sulphate 2 cwt., Mg — Epsom salts 3 cwt., lime — 2 tons ground limestone, compost 10 tons. Trace elements per acre were Mn, Zn, Cu each at 20 lb hydrated sulphates, B as borax 10 lb, Mo as sodium molybdate 5 lb.

Perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*) paspalum (*Paspalum dilatatum*), as well as some crop plants, were raised in the nursery and planted as seedlings in small sub-plots. Samples at grazing height (about 6 in.) were taken, cutting $\frac{1}{2}$ to 1 in. above the crown, and consisted of whole leaves (leaflets plus petioles) in the case of clovers, and leaf tissues in the case of grasses.

Eye estimates of relative growth were made at intervals throughout the growing period and yields were obtained for most crops. Correlation coefficients, significant at 1%, between actual yields and eye estimates indicated that the latter were reasonably reliable. In examples tested in this and in Exp. 2, significant responses calculated from estimated yields were much the same as from actual yield data.

Experiment 2. Horotiu sandy loam, 1947-55. In 1947, the location of the experiment was shifted to near Rukuhia aerodrome on a less fertile soil type, the Horotiu sandy loam, an immature yellow-brown loam from mixed rhyolite and andesite ash alluvium, testing as follows (0–6 in.): pH 5.6; cation-exchange capacity 30 m.e.%; exchangeable cations (m.e.%): calcium 6, magnesium 0.6, potassium 0.4; available phosphorus (Truog) 1.1 mg. % P_2O_5; fixation, very high. In 1948-49, lime was reduced to 5 cwt per acre and potassium chloride was used in place of the sulphate. In 1949-50, lime was increased to 2 tons per acre in an attempt to accentuate the pH differences which up to this time were small (unlimed controls pH 5.6, limed 6.0). In 1950–51, trace elements and magnesium were discontinued. Ammonium sulphate was increased to 4 cwt per acre in the base. A further 2 cwt was applied as a sidedressing on 15 January 1951, because of the wet season. In 1951-52, the superphosphate treatments were increased to 1 ton per acre, potassium chloride to 4 cwt per acre, and borax was applied over all plots at 20 lb per acre. Liming was now discontinued. In 1952-53 and in 1953-54, 5 cwt superphosphate per acre was applied to the control and KN plots (now "low P" plots) and 4 cwt potassium chloride per acre to the controls, but were discontinued in 1954-55. Borax was omitted in 1954. Epsom salts were applied at 5 cwt per acre on the "PKNMg plus trace elements" plots in 1952 and at 10 cwt in 1953 and 1954.

Various plant species were grown, as in Exp. 1.

Experiment 3. Horotiu sandy loam, 1944-45. White clover samples were analysed from a fertiliser trial with a pure stand of clover, conducted by R. K. Ward at Ruakura Animal Research Station in 1944-45. There were four treatments, replicated six times: control, superphosphate at 5 cwt per acre, superphosphate plus lime at 2 tons per acre, superphosphate plus lime plus potassium sulphate at 3 cwt per acre. The latter two treatments were selected for the potassium effect.

Trials with Mixed Pastures

Experiment 4. Rates-of-potassium topdressing trial, Cambridge. On 21 May 1951, a rates-of-potassium experiment was laid down by J. R. Murray, Instructor in Agriculture, Hamilton, on a pasture sown down in March 1950, on a dairy farm near Cambridge, on the Horotiu sandy loam. Soil tests 0–3 in. and 3–6 in. respectively were as follows: pH 6.0, 6.1; cation-exchange capacity 32, 33 m.e.%; exchangeable cations (m.e.%): calcium 12, 10, magnesium 0.7, 0.6, potassium 0.13, 0.12, sodium 0.4, 0.3; available phosphate (Truog) 10, 7 mg % P_2O_5. Grasses were dominantly perennial ryegrass with some browntop (*Agrostis tenuis*) and cocksfoot (*Dactylis glomerata*); clovers were mainly white and red clovers, with some volunteer suckling clover (*Trifolium dubium*). To ensure adequate phosphate, all plots received 6 cwt superphosphate per acre. Lime was not applied, as responses are unlikely in pastures on this yellow-brown loam soil type when the pH is above about 6.0. The potassium treatments were 0, $\frac{1}{4}$, $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2 cwt potassium chloride per acre, applied (a) autumn (21 May) (b) spring (27 August), with two replicates of each treatment. Single additional plots of 4 and 6 cwt of potassium chloride were added to the spring-treated blocks. The area was fenced off to permit quick grazing by dairy cows and thus reduce the transfer of potassium through dung and urine from the high-potassium to the low-potassium plots. In sampling for analysis, obviously contaminated areas were avoided as far as practicable. Estimates were made of relative growth at each sampling. On 17 December 1951, the area was cut with a hay mower, the growth removed, and the paddock closed for five weeks. On 16 January 1952, the area was again mown and yield measurements were taken.

Experiment 5. Rates-of-potassium trials on peat soils. Pasture samples were collected from rates-of-potassium trials laid down on three contrasting peat soils (a) Hauraki Plains peat, an undecomposed peat of very low volume weight and ash content, (b) Rukuhia peat, partly decomposed and intermediate in volume weight and ash content, and (c) Ruakura decomposed loamy peat (Thompson and Elliott 1950).

TABLE 1. Volume Weight and Ash Contents of Peats
(lb per acre 3 in.)

Origin	Dry Peat	Ash
Hauraki Plains	50,000	2,500
Rukuhia	200,000	60,000
Ruakura	350,000	210,000

Analyses were made of separated clovers and grasses from these trials.

Experiment 6. Miscellaneous topdressing trials. Dissected clovers and grasses (mainly ryegrass) collected in 1950 and 1951 were analysed from various trials laid down by Extension officers mainly in the northern half of the North Island. Most samples were taken at cow grazing height. The results of such trials are discussed in generalised articles by Woodcock (1936), Bell (1936, 1937, 1953), Burgess and Lynch (1951), Taylor (1950), Taylor and Bell (1952), and Warner (1953).

Effect of Tissue Analysed on Plant Analytical Levels

To assess the importance of stage of plant development and of specific tissue analysed, selected samples were divided into leaflet and petiole tissues or leaf and stem tissues in the case of legumes and leaf and flower stalk tissues in grasses.

Analytical

All samples were analysed for total nitrogen and phosphorus by chemical methods, potassium, sodium, and calcium by flame photometry, and magnesium and manganese by Lundegardh spectrographic methods. All nutrients other than nitrogen were determined on acid extracts of the ash obtained on ignition at 500°c.

Results

Percentages of potassium (K) in the dry matter, are summarised in Tables 9 to 19. Limited space precludes the presentation of the full data on other nutrients but mean levels are recorded for the potassium-treated plots (PKN in experiments 1 and 2) below most of the tables.

Yield Responses to Fertiliser Application

General. In Exp. 1 and 2, as nitrogen and phosphorus responses were obtained, the only comparison which gives an unambiguous measure of potassium response is PKN compared with PN, hence the reported analytical figures are restricted to those plots. The significant differences referred to in the tables, however, were based on analysis of variance of yield and analytical figures for all treatments in each experiment. Some of the cuts from replicate plots in Exp. 1 and 2 were bulked before analysis. The standard deviations used in determining the significance of the difference in potassium concentrations were from those cuts which were analysed in full. For the composite samples, a reasonable approximation has been obtained by applying the mean coefficient of variation found for the separately analysed samples, rather than the mean standard deviation, as the former is less affected by the size of the mean.

In Exp. 1 and the first season in Exp. 2, potassium sulphate was used, but as there were no responses to this fertiliser, the possibility of sulphur nutrient responses does not arise. Furthermore, sulphur analyses by Steinbergs' (1953) method indicated adequate levels.

In the ryegrass in Exp. 2, there were significant differences between the potassium responses on the limed and unlimed plots in the 1949-50, 1950-51 and 1953-54 seasons. The figures for limed and unlimed plots are therefore kept separate in Table 9. For legumes, the combined results are shown in Table 10.

Experiment 1. There were no potassium responses in grasses or clovers in this experiment and potassium analytical levels were high. The only significant responses were in white clover in the first cut and red and plant analytical levels for other nutrients showed no abnormalities. It is reasonable to assume, therefore, that the growth of the PN and PKN plants was not limited by any deficiency or excess of nutrients and the lack of response to potassium sulphate truly indicated adequate supplies of potassium nutrient.

Experiment 2. Significant superphosphate responses were obtained, some large, especially in clovers after the first season. Significant responses to ammonium sulphate were obtained in perennial ryegrass in some cuts, especially in 1952 and 1954. In spite of heavy annual dressings of superphosphate (10 cwt per acre) applied from 1947 to 1950, there was evidence from similarly conducted rates-of-phosphate experiments that the amount used may have been insufficient for maximum growth on this very high-fixing soil. In some cases, therefore, there is a possibility that potassium responses may have been limited by a mild deficiency of phosphorus. There were no significant trace-element or magnesium responses in clovers and grasses before 1953. The only significant responses were in white clover in the first cut and red clover in the first two cuts in 1953-54. This response is attributed to the magnesium. The available evidence indicates that the growth of the PN and PKN plants has not been appreciably limited by any other nutrient deficiency, with the possible exception that phosphorus may have been in short supply, especially between 1947 and 1950, and magnesium mildly deficient. The grasses may have been slightly deficient in nitrogen in some cuts, in spite of the nitrogen applications.

There were no potassium responses in clovers and grasses (that is differences between PKN and PN were not significant) before the 1950 season. In 1949, visual deficiency symptoms and significant responses to K were noted in potatoes, cabbages, swedes, and mangolds, but the effects on ryegrass were slight and non-significant. In the early part of the 1950 season, some rather variable but significant responses were indicated in perennial ryegrass, but these were not sustained in subsequent growth during that same season. In the following season the effects were highly significant in the early season, but again were not apparent later in the season. Clovers were not included in the 1949 and 1950 seasons, but in 1951 highly significant responses were noted in white clover, associated with marked deficiency symptoms in the PN plots, and similar effects, though less severe, were observed in subterranean clover (var. Tallarook) in the 1952 season. In 1953-54, in spite of a high coefficient of variation, Italian ryegrass and the second cut of white clover showed significant responses, but although the red clover (broad red) showed distinct deficiency symptoms in many plants in the PN plots, the responses did not reach significance. In the 1954-55 season, significant responses were found in lucerne, perennial ryegrass, cocksfoot, and Yorkshire fog (*Holcus lanatus*), but not in sweet vernal (*Anthoxanthum odoratum*) or paspalum.

Experiment 3. Increased yields from potassium sulphate over and above the lime and phosphate effects were obtained at each cut from this experiment, but not all the differences reached significance. The March 1944-March 1945 result in Table 11 was for a weighted composite of eight cuts taken at approximately monthly intervals. Five showed no significant potassium responses, two were significant at 5%, one at 1%. Overall, the response nearly reached significance at the 5% level.

Experiment 4. This experiment was laid down as an observational topdressing trial, consequently the layout was not randomised, but the results were so outstanding as to make statistical analysis unnecessary. Responses from the autumn applications first appeared as increased clover growth in September (spring), and by the middle of October marked responses were evident on both the autumn- and spring-topdressed blocks. At the first plant sampling for analysis at the beginning of October, the autumn-treated plots were showing more pronounced responses than the spring-treated, but by the end of October there was little difference. At 2 and 29 October 1951 the spring 2K (2 cwt KCl per acre) plots were superior to the plots with smaller dressings and equal to the 4K, but after November the 4K plot was better than any of the 2K plots. Optimum growth was therefore at the 2 cwt rate for the October samplings and at the 4 cwt rate (or between 2 and 4 cwt) for December and February samplings and the "hay cut" in January. Average dry-matter yields per acre for the 5½ weeks growth period from 7 December 1951 to 16 January 1952 were as follows: Nil potassium chloride 8½ cwt; ¼ cwt 12; ½ cwt 13; 1 cwt 16; 1½ cwt 20; 2 cwt 22; 4 cwt 30½; 6 cwt 24 cwt. This cut included growth from dung and urine patches and this disproportionately increased yields from the poorer plots. The lower yield for the 6 cwt treatment compared with 4 cwt may have been due to the extreme dominance of clover growth, over 90% at this stage. Later the 6K treatment appeared to be best. Both the 4K and the 6K plots remained clover dominant till after May 1952. By February 1953, grasses were more than 50% in these plots.

Nine series of samples were taken from the experimental plots, dissected into mixed grasses, white clover, and red clover, and analysed with the results shown in Tables 12 to 14. Suckling clover was also separated in the cut of 29 October (Table 15). Results are shown for the spring-topdressed plots only. Because of the difficulty in obtaining samples from the poorest plots, the clover results may be biassed towards higher levels in the low potassium plots, the worst parts of which were practically devoid of clovers.

Experiment 5. On the wet Hauraki Plains peat (Table 16), responses were obtained to all increments of applied potassium chloride, and it is possible therefore that the highest rate may have been inadequate owing to losses through leaching or surface run off.

Experiment 6. Tables 17 and 18 show results of analysis of clover, *Lotus* sp., and grass samples from observational field plots with and without added potassium. Clear evidence is lacking whether additional responses would have been obtained from further applications of potassium chloride, though two rates were used in some trials, or whether smaller quantities would have sufficed. The figures for samples from the potassium-treated plots therefore cannot be regarded as necessarily adequate. Where clear responses have been obtained, however, the figures for the control plots can certainly be accepted as deficient levels. (Note: A definite yield response in observational trials under grazing can only be accepted when there is a clear line of demarkation, due to improved growth, between the control and treated plots.)

Samples from further trials on responsive soils, from which clovers only were dissected out and analysed, showed similar levels.

Soil Analytical Levels

The depleting effect of the exploitive cropping system used in Exp. 2 (all growth was removed), is apparent from the soil potassium levels which fell from 0.4 m.e. % exchangeable potassium in the top 6 in. in 1947 to 0.15 m.e.% in the PN plots by the end of the 1949-50 cropping season. Levels remained between 0.15 and 0.12 m.e.% in subsequent years. The PKN plots, by contrast, showed exchangeable-potassium levels of more than 0.4 m.e.%.

The soil from the very deficient area in Exp. 4 on the same soil type showed 0.13 m.e.% K and this may be an "equilibrium" depletion level (Bray and De Turk 1938) for the Horotiu sandy loam, representing a balance between removal of exchangeable potassium by plant roots and replenishment from non-exchangeable forms.

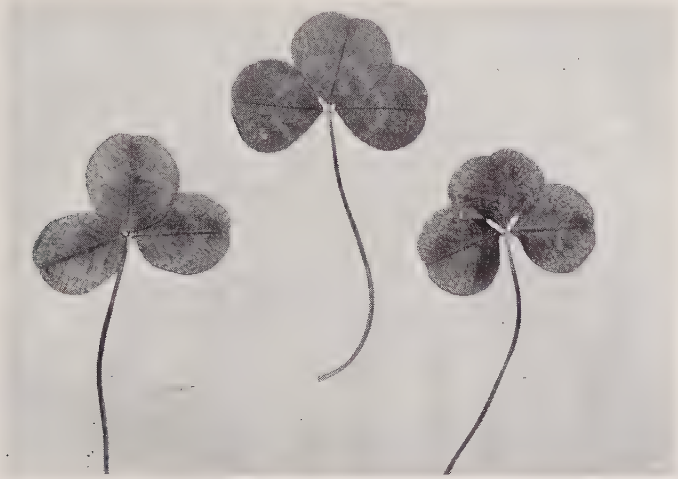


Fig. 1.—Symptoms of potassium deficiency in white clover (top leaf normal).

Deficiency Symptoms

Typical symptoms in white-clover leaves are illustrated in Fig. 1. Symptoms first appear on the mature leaves, the youngest remaining healthy. The most characteristic effect is the appearance of a pattern of small white spots near the margins of the leaflets. Later this develops into a marginal leafburn and the affected leaves die. This premature death of the older leaves is common, especially at flowering and in drier weather following good growing conditions. Sometimes, especially during winter months, there are no obvious symptoms apart from small size of leaves.

In red clover, the early symptoms are similar to those in white clover, giving a pattern of rather more closely spaced, oval spots, usually pale brown. The outer tissues become bronzed or brownish in colour, and, as these tissues die, the colour darkens. At this later stage the leaf margins often assume a very ragged appearance. The symptoms noted agree well with those described by Paton (1950) in Tasmania. As with white clover in an early stage of growth, the youngest leaves may show no symptoms, while older leaves on the same plants are badly affected. Potassium levels in one typical example were 0.61 and 0.33% respectively. Later, however, with the development of the flowers, the drain on potassium may be sufficient to cause severe symptoms to develop even on the youngest leaves on the flower stalk.

In Tallarook strain of subterranean clover, the first symptoms noted were a slight bronzing of the marginal tissues and this soon developed into a pale brown marginal chlorosis sometimes accompanied by necrotic spotting near the margins. Some die back of the margins of the leaflets was noted as the chlorosis extended inwards. The symptoms appear to be similar to those described by Rossiter (1947) and Millikan (1953) for the Dwalganup and Mt. Barker strains.

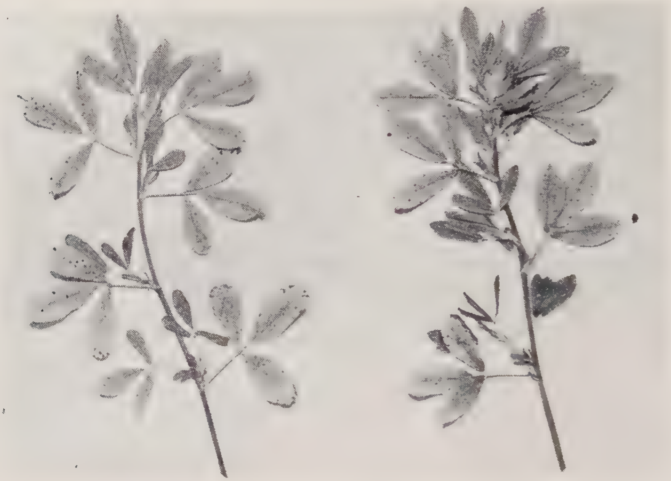


Fig. 2.—Symptoms of potassium deficiency in lucerne.

In suckling clover (*Trifolium dubium*) and lucerne (Fig. 2) the pattern of white spots is similar to that of white clover, but the symptoms are usually more distinct in leaves near the top of the stem, the lower leaves frequently showing only a chlorosis of the outer parts of the leaflets, often accompanied by die back of the leaf tips.

These symptoms in legumes are like those described by Richards and Templeman (1936) as characteristic of conditions of potassium deficiency in conjunction with relatively low phosphorus and sodium and high calcium levels.

Potassium-deficiency symptoms in grasses were difficult to identify. A few plants in PN plots in Exp. 2, showing chlorosis of the top inch or tip burn of the older leaves, gave a potassium figure of 0.25%, and this was probably a true case of potassium-deficiency symptoms. In other cases either there were no clear symptoms or they were difficult to distinguish from the effects of nitrogen deficiency or fungal injury or drought and senescence.

Exp. 4 showed typical seasonal incidence of deficiency symptoms in white and red clovers in mixed pastures subject to rotational grazing. Symptoms were first noted in white clover during the spring flush early in October 1951, especially on the control plots and headlands. Some plants were affected on the $\frac{1}{4}$ and $\frac{1}{2}$ cwt KCl plots and a lesser number on the 1 cwt KCl plots. By the end of October, red clover and suckling clover were showing symptoms. At this stage spotting symptoms in white clover were most conspicuous on the $\frac{1}{4}$ and $\frac{1}{2}$ cwt KCl plots, then 1 cwt KCl and the stunted control, while the 2 cwt KCl plots were nearly free. No symptoms were noted on any white clover plants on the 4 and 6 cwt KCl plots, nor in red clover on the 6 cwt KCl treatment at any stage. In January 1952, symptoms were still visible on white and red clovers, but by the end of February were slight in white clover, though there was an appreciable marginal leaf-burn in red-clover leaves. A few affected red-clover plants were noted even on the 4 cwt KCl treatment, presumably on spots which missed the fertiliser application (confirmed also by analytical levels). From March to May, growth was restricted through dry weather, and responses were slight. No clear symptoms were noted in the May sampling nor in the late winter sampling in August. Symptoms again appeared in white clover with the spring flush in October 1952.

Effect of Tissue Analysed, and Stage of Growth, on Potassium Levels

Leaflet versus petiole in legumes. The results for clovers reported in the Tables were for whole leaves (i.e. leaflets plus petioles). The following figures are typical of the conspicuous and consistent trend towards higher potassium levels in the petioles than in the leaflets, especially in plants adequately supplied with potassium:

TABLE 2. Per Cent K in Dry Matter
(Subterranean and white clover)

Tissue	Subterranean Clover			White Clover		
	K Deficient (PN)		Healthy	K Deficient	Healthy	
	With Symptoms	No Symptoms	PKN	10P 1K	10P 2K	10P 5K
Leaflet	0.41	0.71	2.41	0.87	1.61	2.51
Petiole	0.47	0.95	4.57	1.30	2.45	4.71
Whole leaf	0.42	0.76	2.98	1.03	1.97	3.30

Petioles show a greater sensitivity to potassium changes than leaflets, an effect also noted by Ulrich (1946) in Ladino white clover. Under grazing conditions, however, it is often difficult to secure suitable samples of clovers for separation of petiole and leaflet for analytical work, and therefore combined leaflet plus petiole samples have been used in this investigation and can be employed in diagnostic or research work without serious loss of sensitivity.

In severely potassium-deficient plants, the differences in potassium levels between leaf blade and petiole tissues may be slight, but at higher levels, including the threshold or critical range, the difference is greater. Therefore, in the case of clovers with a squat habit, both height of pasture growth, and therefore length of petiole, and height of sampling in the field can detectably affect the apparent nutrient status of the clover plants when whole leaves are analysed.

Leaf versus stem tissue in legumes. In red clover, with an erect habit, samples at grazing height, and cut from about 1 in. above ground level, may include some stem tissue as well as leaflets and petioles. In some legumes, such as lucerne, samples always include stem tissue. In red clover or in lucerne, the ratio of stem to leaf tissue increases with maturity.

In red clover at a relatively young stage of growth, similar levels of potassium are found in petiole and stem tissues and higher levels in these tissues than in the leaflets or whole leaves, especially in healthy plants, as in the following examples cut at 9–12 in.

TABLE 3. Per Cent K in Dry Matter
(Red clover)

	Leaflet	Petiole	Whole Leaf	Stem	Whole Top Growth
K deficient (PN)	0.78	1.09	0.86	1.01	0.88
Healthy (PKN)	2.55	5.22	3.38	4.63	3.58

At the hay stage, however, the stem tissue in red clover may have a lower potassium concentration than the whole leaves. This may be related to the increased lignification of the basal stem tissues, as the succulent top 6 in. growth still shows a distribution of potassium characteristic of immature growth, namely much higher potassium in the stem than in the leaves in healthy plants. At intermediate growth stages, with corresponding crude-fibre values, intermediate potassium distributions are found.

TABLE 4. Effect of Stage of Growth on Potassium Contents of Leaf and Stem Tissues of Healthy Red Clover

	Plant Height (in.)	Per Cent K in Dry Matter			Per Cent Fibre in Whole Growth
		Leaves	Stem	Whole Growth	
Whole growth	9–12	3.18	4.08	3.45	22
,, ,,	12–18	3.34	3.70	3.47	27
,, ,,	20–24	3.06	2.80	2.90	33
Top 6 in.	20–24	2.90	4.71	3.29	
Remaining 14–18 in.	20–24	3.24	2.62	2.77	

Effects similar to those in red clover are also found in lucerne, but here the change in distribution of potassium between leaf and stem tissues with age commences at a relatively young growth stage. In potassium-deficient lucerne the trend towards similar levels in both stem and leaf tissues is seen even in plants only 6 to 9 in. tall.

Wallace *et al.* (1948) found similar results in lucerne whose potassium status at the early blossom stage (about 1.2% K) was described as near the critical level. The concentration of potassium

TABLE 5. PER CENT K IN DRY MATTER OF K-DEFICIENT LUCERNE

Plant Height (in.)	Leaves with Symptoms	Healthy Leaves, Tips, Laterals	All Leaves	Stem	Whole Growth
9	0.80	1.37	1.07	1.03	1.05

In taller plants the top 6 in. of lucerne has a higher potassium status than the basal tissues.

TABLE 6. PER CENT K IN LUCERNE PLANTS OF DIFFERENT HEIGHTS

Sample	Plant Height (in.)	Top 6 in.	Remaining Tissues	Whole Growth
K deficient	12–15	0.89	0.51	0.72
Healthy	12–18	2.06	1.48	1.74

in the top 3 in. was approximately 2% compared with 1% for the remaining tissues.

Clearly, sampling height is important in legumes with a tall habit and can have a profound effect both on the analytical levels found and on their interpretation.

If the top 3 to 6 in. of a legume conserved for hay is taken for analysis to ensure a more uniform stage of growth, it is still practicable to select specific tissues for analysis and here again the choice of tissue may profoundly affect the analytical level obtained.

TABLE 7. POTASSIUM CONTENT OF DIFFERENT TISSUES DISSECTED FROM THE TOP 6 IN. GROWTH OF LUCERNE

Sample	Plant Height (in.)	Leaves	Tips and Laterals	Stem	Whole Top 6 in.
Healthy	18	1.79	2.35	5.34	2.69
K deficient*	10	0.44	0.99	1.24	0.86

*Leaves showing typical deficiency symptoms were selected from potassium-deficient plants. "Tips and laterals" included all the younger leaves free from symptoms.

A higher status and minimum requirement is indicated for the whole top 6 in. than for the leaf component of this sample. The contrast between stem and leaf tissues is still greater.

Leaf versus flower-stalk tissue in grasses. In grasses at grazing height before flower emergence, there is little difference in potassium levels between the bottom inch (young growth) and the top inch (older growth) of the leaves. Lenience of cutting, at this stage, therefore has little effect on the analytical levels.

At flower emergence, however, the stalk tissue stripped of leaves has a lower concentration of potassium than the leaves in plants well supplied with this nutrient, but may be slightly higher in potassium-deficient plants. This is illustrated by results for Italian ryegrass from potassium-deficient (PN) and adequate (PKN) plots.

TABLE 8. PER CENT K IN DRY MATTER
(Italian ryegrass)

		Vegetative Growth	At Flower Emergence		
			(a) Leaf	(b) Stalk	(a) and (b) Combined
K deficient, Plot	A	1.10	0.74	1.14	0.98
	B	0.70	0.70	1.00	0.91
Healthy	C	4.05	4.23	3.00	3.50
	D	4.72	4.62	2.86	3.49

In the first column are shown results for plants before flower emergence, while the next three columns refer to more advanced plants showing clear development of the flower stalk and taken at the same time from the same plots.

The potassium levels are higher in the stalk tissue than in the leaves in the deficient plants and the reverse in healthy plants. Thus the trend of results is similar to that of red clover and lucerne at the hay stage.

DISCUSSION

Seasonal Variation in Potassium Content

Potassium levels tended to be lower in summer and autumn in Exp. 2 and 4. A marked seasonal decline in potassium concentration has many times been demonstrated in legumes and grasses grown to maturity. Evidence such as that of Woodman *et al.* (1933) with lucerne cut at intervals for hay indicates that this seasonal effect is dominantly a stage-of-growth effect, as the pattern of changes in potassium levels is repeated in the new growth after each cut. With pastures under grazing, the growth interval is small and successive samples represent mainly new growth, commencing each time from the date of the previous grazing. Differences in stage of growth should be small and samples relatively comparable at each sampling date. In practice, however, samples taken for analysis from pastures under grazing may range from about 2 to 10 inches of height, with resulting differences in stage of development of the tissues.

In Exp. 4, normal samples taken between grazings ranged in height between about 3 and 8 in., while the January "hay cut" represented $5\frac{1}{2}$ weeks growth. During the hot, sunny, and dry weather of autumn, when growth was retarded and the intervals between grazings were longer, plants tended to mature. Such conditions also favour lignification of tissues and carbohydrate synthesis, which has a "dilution" effect in lowering plant cation concentrations (Wallace and Bear 1949).

In Tables 12 to 14 the minimum levels for samples from the potassium-treated plots were in the late autumn cuts (May). This temporary drop after autumn rains may be due in part to water washing of the foliage which has been shown by various workers (reviewed by Mes 1954) to lower the potassium content of the foliage. Other possible factors are drain on foliage potassium for changes in root development and increases in depth of roots as observed by Jacques (1956) in summer and autumn; or changes in the amount of available potassium with alternate wetting and drying of the soil (Walsh and Cullinan 1945; Luebs *et al.* 1956).

Correlation of Plant Analytical Levels with Fertiliser Applications

In general, the results show increased concentrations of potassium from applications of potassium fertilisers. The contrast between treated and untreated plots is most marked where the control plots are clearly deficient and the treated plots over-supplied with potassium fertiliser (e.g. Table 10, Exp. 2, 1951-2). On the other hand, where the potassium status is already very high, application of potassium fertiliser may have no detectable effect on uptake (e.g. Table 10, Exp. 1).

Correlation of Deficiency Symptoms with Potassium Levels

In all cases except one, deficiency symptoms in clovers were associated with potassium levels below 0.7% in affected whole leaves. The exception, 0.87% K, was for one sample of white-clover leaves with spotting symptoms from vigorously growing plants with abnormally long petioles (paddock closed for hay). Figures have been obtained down to 0.6 % for apparently healthy young leaves from plants showing severe symptoms in older leaves, but usually "healthy" foliage is higher in potassium content. Rossiter (1947) working with Dwalganup subterranean clover reported that potassium-deficiency symptoms may be expected when the potassium content of either leaf (leaflet) or leaflet plus petiole falls below 0.8%. Chandler *et al.* (1945) working with Ladino white clover at the early-bloom stage, reported that symptoms of potassium deficiency appeared on clover leaves when the potassium content of the whole above-ground growth of plants fell below 0.8% on the oven-dry basis. A consideration of the evidence presented on the effects of tissue analysed suggests that this figure of 0.8% for healthy plus affected leaves would correspond to about 0.6% to 0.7% if only affected leaves had been analysed.

Table 19 shows the range of values and the average potassium levels for samples at grazing height from plants (a) with potassium-deficiency symptoms, (b) showing significant responses to applied

TABLE 9. EXP. 1 & 2. MEAN CONTENTS OF POTASSIUM IN GRASSES
(Range of values in parentheses)

Species Season and Dates	Relative Yields‡ (%)		No. of Cuts	K % in dry matter			
				PN		PKN	
	Unlimed	Limed		Unlimed	Limed	Unlimed	Limed
Perennial ryegrass—							
Exp. 1 1946-47 (Jan.-June 1947)	95	96	4	4.54 (4.15–5.01)	4.47 (4.22–4.82)	4.67 (4.35–5.19)	4.52 (4.21–4.80
Exp. 2 1947-8 (Dec. 1947-June 1948)	96	104	5	3.29 (2.86–3.65)	3.12 (2.86–3.40)	3.20 (3.02–3.50)	3.25 (2.82–3.88
1948-9 (2 Feb. 1949)	107	94	1†	2.32	2.03	3.61**	3.75**
1949-50 (17 April 1950)	89	136**	1†	1.64	1.27	1.86	2.50**
(June & July 1950)	85	118	2†	1.95 (1.51–2.38)	1.73 (1.44–2.01)	2.75** (2.40–3.09)	2.69** (2.05–3.32
1950-1 (28 Nov. 1950)	150*	180**	1	0.86 (0.72–1.07)	1.21 (1.08–1.36)	3.45** (3.29–3.62)	2.94** (2.87–3.00
(Feb. & June 1950)	127	113	2	1.09 (0.83–1.60)	1.42 (1.12–1.72)	2.26** (1.65–3.40)	1.98* (1.57–2.77
1952-3 (9 Dec. 1952)	144**	159**	1	0.85 (0.76–0.95)	0.94 (0.77–1.05)	3.58** (3.28–3.89)	3.23** (2.92–3.48
1954-5 (Dec. 1954-Feb. 1955)	149**	187**	3	0.76 (0.61–0.97)	0.63 (0.43–0.78)	4.80** (4.71–4.92)	4.01** (3.48–4.46
Italian ryegrass (Dec. 1953-Jan. 1954)	139	165*	2	1.33 (1.17–1.67)	0.95 (0.78–1.10)	3.75** (3.30–4.34)	3.95** (3.41–4.78
Yorkshire fog (20 Dec. 1954)	218*	221*	1	1.34 (0.97–1.88)	1.12 (0.96–1.29)	4.84** (4.63–5.13)	4.48** (4.40–4.54
Cocksfoot (17 Jan. 1955)	329**	361**	1	0.94 (0.83–1.14)	0.88 (0.77–0.99)	4.41** (4.22–4.71)	4.07** (3.85–4.26
Paspalum Exp. 1 (26 Feb. 1947)	85	92	1	3.09 (2.90–3.28)	2.96 (2.60–3.34)	3.06 (2.86–3.37)	3.09 (2.92–3.31
Exp. 2 (Jan. & Mar. 1955)	78	97	2	0.41 (0.28–0.48)	0.45 (0.34–0.53)	2.80** (2.27–3.36)	2.62** (2.27–3.23
Sweet vernal (11 Jan. 1955)	71	130	1	1.23 (1.14–1.33)	0.90 (0.83–0.97)	4.42** (4.33–4.47)	3.97** (3.73–4.25
Flowering (12 Oct. 1955)	110	103	1	1.31 (1.29–1.33)	1.16 (1.13–1.20)	2.51** (2.25–2.71)	2.60** (2.40–2.79

‡ Yields of PKN relative to PN treatment (100%).
† Composites of the 3 plots in each treatment.
* Differences (PKN-PN) significant at 5%. ** Significant at 1% or better.

Mean status of other nutrients (per cent dry matter, or ppm Mn, in PKN plants).

Perennial ryegrass:	N	P	Na	Mg	Ca	Mn
Exp. 1:	3.76	0.25	0.08	0.20	0.71	133
Exp. 2: (1947-51)	3.31	0.20	0.30	0.24	0.72	104
(1952-3)	3.20	0.30	0.30	0.13	0.76	68
(1954-5)	3.20	0.24	0.23	0.20	0.60	142
Italian ryegrass	2.97	0.25	0.12	0.16	0.65	128
Yorkshire fog	3.76	0.32	0.13	0.14	0.51	142
Cocksfoot	3.16	0.23	0.18	0.17	0.38	123
Paspalum						
Exp. 1:	2.38	0.22	0.02	0.16	0.27	118
Exp. 2:	2.37	0.16	0.02	0.16	0.49	88
Sweet vernal (11.1.55)	3.03	0.27	0.09	0.03	0.42	135
Flowering (12.10.55)	1.73	0.29	0.03	0.05	0.29	165

TABLE 10. Exp. 1 & 2. Mean Contents of Potassium in Clovers and Lucerne (Range of values in parentheses)

Species Season and Dates	Relative Yields %	No. of Cuts	K % in Dry Matter	
			PN	PKN
White clover				
Exp. 1 1946-7 (Feb. to June 1947)	109	3	3.90 (3.34–4.30)	3.90 (3.18–4.34)
Exp. 2 1947-8 (March & May 1948)	90	2	3.21 (2.98–3.40)	3.16
1948-9 (Dec. 1948-Aug. 1949)	98	5	2.47 (1.43–3.38)	3.38* (2.33–3.83)
1951-2 (Dec. 1951 & Jan. 1952)				
(a) Normal sample‡	246**	2	0.79 (0.62–0.99)	3.02** (2.08–3.83)
(b) With symptoms		2	0.49 (0.29–0.66)	
1953-4 (18 Jan. 1954)	96§	1	0.55 (0.47–0.60)	2.84** (2.58–3.10)
(29 March 1954)	155**	1	0.97 (0.78–1.13)	3.61** (3.24–3.93)
Subterranean clover				
Exp. 2 1947-8 (3 Feb. 1948)	109	1†	3.06	3.31
1948-9 (21 Dec. 1948)	100	1†	1.48	2.32**
1952-3 (Dec. 1952 & Jan. 1953)				
(a) Normal sample‡	240**	2	0.61 (0.46–0.76)	2.49** (2.20–3.08)
(b) With symptoms		2	0.42 (0.37–0.47)	
Red Clover 1953-4 (Dec. 1953-March 1954)	109	3	1.00 (0.77–1.55)	3.23** (2.40–3.86)
Flowering, Feb. 1954	110	1	0.62 (0.48–0.94)	2.26** (1.76–2.62)
Lucerne, 26 Jan. 1955	296**	1	0.56 (0.45–0.74)	2.05** (1.70–2.31)

See footnote to Table 9

† Composites of the 6 plots in each treatment. § Affected by dry weather.

‡ Normal sample from the PN plots included young and older leaves, frequently with deficiency symptoms.

Mean status of other nutrients (% D.M. or ppm Mn, in PKN plants):

	N	P	Na	Mg	Ca	Mn
White clover						
Exp. 1	3.97	0.25	0.07	0.20	1.15	110
Exp. 2 (1947-9)	5.02	0.28	0.11	0.20	1.46	75
(1951-2)	4.17	0.23	0.13	0.19	1.87	79
(1953-4)	3.90	0.26	0.16	0.17	1.69	132
Subterranean clover						
(1947-9)	3.75	0.20	0.37	0.20	1.89	129
(1952-3)	4.00	0.24	0.21	0.12	1.30	63
Red clover, Normal	3.94	0.27	0.09	0.33	1.91	107
Full flowering	3.04	0.21	0.07	0.31	1.87	68
Lucerne, 26.1.55	2.92	0.22	0.07	0.18	1.92	48

potassium, (c) uncertain, (d) potassium-treated, (e) from non-responsive soils. Section (c) includes samples from cuts showing no significant responses to potassium fertiliser but taken from plots showing significant responses in earlier or later cuts in the same season.

Correlation of Plant Analytical Levels with Responses to Applied Fertilisers—Critical or Threshold Levels

General. The relationship between yield and nutrient concentration in plant tissues has been reviewed especially by Goodall and Gregory (1947), Ulrich (1948), and Hewitt (1950).

Ulrich (1943) defined the "critical nutrient concentration" of a plant as "that narrow range of concentrations at which the growth rate or yield first begins to decrease in comparison to plants at a higher nutrient level".

The critical level is determined from results of pot or field experiments in a deficient medium, using increasing rates of application of the specific nutrient under study. All elements other than this nutrient should be present in the culture or be added to the soil in amounts sufficient for maximum growth. The usual method is then to plot the nutrient concentrations in the selected plant tissues (horizontal axis) against yields (vertical axis). If an acutely deficient medium is used, and growth conditions are suitable, this normally results in a curve made up of three parts, a steeply ascending branch or deficiency zone where yield increases though internal concentration remains relatively constant, a transition zone in which both yield and internal concentration increase, and finally a horizontal branch or zone of adequacy in which yields remains constant but internal concentrations

TABLE 11. EXP. 3. MEAN CONTENTS OF POTASSIUM IN WHITE CLOVER
(Range of values in parentheses)

Date	Growth Interval	Relative† Yields (%)	K % in Dry Matter	
			LP	LPK
March 1944 to March 1945	Weighted composite	109.4*	2.55 (2.44–2.65)	(2.94–3.34)
23 April 1945	1 month (composite)	127.4**	2.09	3.33**
21 Sept. 1945	5 months	124.3*	2.09 (1.96–2.22)	3.22** (2.84–3.44)
19 Nov. 1945	1 month	104.5	1.43 (1.30–1.52)	2.33** (2.14–2.49)

† Yields of LPK relative to LP (100%).

* Differences (LPK–LP) significant at 5%. **Significant at 1% or better.

Average status of other nutrients (per cent dry matter, or ppm Mn, in PKN plants):

N 5.31 P 0.36 Na 0.14 Mg 0.19 Ca 1.59 Mn 56

TABLE 12. Exp. 4. Potassium Content of White Clover from Rates-of-Potassium Experiment
(K % in dry matter)

		Yield† 16.1.52	2.10.51	10.10.51	29.10.51	25.11.51	16.1.52	25.2.52	28.5.52	25.8.52	8.10.52	Mean	Corrected Means	Diff. from Control
Control	N‡ S	8.4	0.76	0.91 0.38	0.65 0.34	1.31 0.35	0.89 0.35		0.89	0.98	1.24	0.95 0.36	0.91	
½ cwt KC1	N S	13.0		1.36 0.54	0.72 0.29	1.06	1.07	0.88	0.66			0.96 0.42	0.99	0.08
1 cwt KCl	N S	15.5	1.51	1.95 0.49	1.04 0.46		0.92 0.39	0.95	0.94	1.24	1.87	1.30 0.45	1.33	0.42*
2 cwt KC1	N S	21.9	2.48	2.38	2.10	1.86	1.01 0.47	1.05	1.19	1.51	2.27	1.76 0.47	1.76	0.85**
4 cwt KC1	N	30.7					2.37	1.97	2.00	1.35	2.17	1.97	2.14	1.23**
6 cwt KC1	N	24.4			3.07		3.05	2.30	2.23	2.08	2.52	2.54	2.68	1.76**

† Total yield of mixed herbage (lb dry matter per acre for period 7.12.51 to 16.1.52). Values underlined indicate the treatments giving maximum growth of clovers at each sampling date.

‡ "N" refers to normal healthy foliage, "S" to leaves showing potassium deficiency symptoms.

* Significant at 5%, ** at 1%. Next to last column shows expected mean values after allowing for missing samples.

Average status of other nutrients in 2 cwt KC1 plots (per cent dry matter, or p.p.m. Mn):

N 5.68 P 0.42 Na 0.56 Mg 0.24 Ca 1.80 Mn 72

TABLE 13. EXP. 4. POTASSIUM CONTENT OF RED CLOVER FROM RATES-OF-POTASSIUM EXPERIMENT
(K % in dry matter)

		Yield 16.1.52	2.10.51	10.10.51	29.10.51	26.11.51	16.1.52	25.2.52	28.5.52	25.8.52	8.10.52	Mean	Corrected Means	Diff. from Control
Control	N	8.4	0.75	0.74	0.60	0.75	1.04		0.63		1.41	0.85	0.81	
	S			0.47	0.32		0.49					0.43		
½ cwt KC1	N	13.0		1.30	0.78	1.07	1.05	0.78	0.63			0.94	1.01	0.26
	S				0.34		0.57	0.28				0.40		
1 cwt KC1	N	15.5	1.77	1.99	1.15		0.99	0.84	0.77	1.26	2.15	1.37	1.38	0.57*
	S				0.43		0.51	0.28				0.41		
2 cwt KC1	N	21.9	2.58	2.42	2.25	2.17	1.09	1.12	0.86	1.50	2.09	1.79	1.79	0.98**
	S						0.53	0.28				0.41		
4 cwt KC1	N	30.7					1.89	2.05	1.56	1.49	2.34	1.87	2.05	1.24**
	S							0.64				0.64		
6 cwt KC1	N	24.4			3.05		2.40	2.37	1.97	2.02	2.46	2.38	2.52	1.71**

(See footnote Table 12)

Average status of other nutrients in 2 cwt KC1 plots (per cent dry matter, or p.p.m. Mn):

N 5.48 P 0.38 Na 0.27 Mg 0.33 Ca 1.84 Mn 79

TABLE 14. EXP. 4. POTASSIUM CONTENT OF GRASSES FROM RATES-OF-POTASSIUM EXPERIMENT
(K % in dry matter)

	Yield 16.1.52	2.10.51	10.10.51	29.10.51	26.11.51	16.1.52	25.2.52	28.5.52	25.8.52	8.10.52	Mean	Corrected Mean	Diff. from Control
Control	8.4	1.20	1.43	1.18	1.12	1.51		1.56	1.68	1.51	1.40	1.41	
½ cwt KC1	13.0		2.11	1.83	2.28	2.11	1.84	1.10			1.88	1.80	0.39
1 cwt KC1	15.5	1.97	2.87	2.29		1.86	1.90	1.18	1.73	2.41	2.02	2.08	0.67*
2 cwt KC1	21.9	2.61	2.99	2.83	3.49	2.63	2.10	1.42	1.75	2.45	2.47	2.47	1.06**
4 cwt KC1	34.7					3.12	2.92	2.17	1.58	2.92	2.54	2.70	1.29**
6 cwt KC1	24.4			2.99		3.60	3.86	2.83	2.72	3.11	3.18	3.26	1.85**

(See footnote Table 12)

Average status of other nutrients in 2 cwt KC1 plots (per cent dry matter, or p.p.m. Mn):

N. 3.2   P 0.47   Na 0.62   Mg 0.21   Ca 0.90   Mn 97

TABLE 15. Exp. 4. Potassium Content of Suckling Clover (*Trifolium dubium*) from Rates-of-Potassium Experiment, 29.10.51
(K % in dry matter)

Control		½ cwt KCl		1 cwt KCl	2 cwt KCl	6 cwt KCl
Symptoms	Normal	Symptoms	Normal	Normal	Normal	Normal
0.43	0.60	0.39	0.75	1.00	1.63	2.31

increase. Macy (1936) referred to these sections of the curve as the "minimum percentage", "poverty adjustment" and "luxury consumption" zones respectively. The transition from poverty adjustment to luxury consumption takes place at the "critical percentage". On this definition the critical level is the minimum concentration in plant tissue for maximum growth.

In determining critical levels, some workers have been influenced by practical considerations of the economics of fertiliser applications. It is clearly necessary to distinguish between physiological critical levels, such as Ulrich's or Macy's values, and economic criteria or "critical levels", such as Lundegardh's (1951) "index values" where the figure will be influenced by profitability of a fertiliser application. Furthermore it is important to distinguish between critical levels obtained by relating plant nutrient concentrations to yields *at the time of sampling for analysis,* and "anticipatory" critical levels such as Lundegardh's and Nicholas's (1952), where the plant tissues are sampled for analysis well in advance of yield measurements. Again it is necessary to distinguish between critical levels in relation to yield and critical levels in relation to the appearance of deficiency symptoms.

The objective of the present work was primarily to determine the physiological critical level, in relation to yield, from field trials, as a basis for interpretation of results of plant analyses. Results from field trials, however, tend to give more extended transition zones and consequently less-certain critical values. To allow for this uncertainty from field trials with their associated high experimental error, some workers have used least significant differences. Yield increases following fertiliser applications (instead of yields) are plotted against plant-nutrient concentrations. A line is then drawn parallel with the horizontal axis at the minimum yield increase required for statistical significance. The point where this line cuts the curve relating responses to nutrient levels is taken as the critical level. In special cases, as applied for example by Chandler *et al.* (1946), this method has particular merit, but as a general technique it is unsatisfactory as the criteria will depend on the experimental error. "Critical" values will be relatively high when plot variability is small and relatively low when the experimental error is large. For the above reasons the nutrient concentrations in the plant tissues at about 90% of the apparent maximum yields have been used in the present work, rather than those values associated with minimum yield responses for statistical significance.

TABLE 16. Exp. 5. POTASSIUM CONTENTS OF LEGUMES AND GRASSES FROM POTASSIUM EXPERIMENTS ON PEAT SOILS
(K % in dry matter)

	Treatments	White Clover	Red Clover	*Lotus* sp.	Grasses	Yield Responses
Hauraki Plains 14.11.48	6P0K	0.41 (symp)		0.33 (symp.)	0.87	Responses at all increments
	6P1K	0.85		0.50	1.41	
	6P2K	0.83		0.67	1.45	
	6P3K	1.16		0.82	1.60	
	6P4K	1.22		0.88	1.71	
	6P6K	1.47		1.29	1.68	
	6P8K	1.76		1.21	1.87	
17.12.48	6P0K				1.40	
	6P3K	1.54			2.30	
	6P6K	2.00			2.15	
Rukuhia 19.10.50	2P0K*	0.42 (symp.)				Responses to ½ and 1K
		0.72 (no S.)	0.51			
	2P½K	1.82	2.09	1.73	2.34	
	2P1K	2.64	2.74	2.50	2.32	
	2P2K	3.27	3.27	3.10	2.85	
	4P2K	3.82	3.69	3.57	3.67	
1.12.50	2P0K*	2.12	2.09	1.65	2.54	No K response at this sampling
	2P½K	2.12	2.11	1.92	2.92	
	2P1K	3.11	3.28	2.60	3.80	
	2P2K	3.50	3.78	3.10	4.10	
	4P2K	3.21	3.32	2.97	4.10	
Ruakura 10.12.47	10P0K	0.88				Responses to all increments
	10P½K	0.96				
	10P1K	1.03				
	10P2K	1.97				
	10P5K	3.30				

* Following 2 cwt superphosphate plus 1 cwt KCl (2P1K) in September 1950.

Average status of other nutrients in best plots (per cent dry matter, or p.p.m. Mn):

	N	P	Na	Mg	Ca	Mn
Hauraki Plains, 6P8K:						
White clover:	4.14	0.27	0.10	0.23	2.28	101
Lotus sp:	3.00	0.23	0.14	0.29	2.40	115
Ryegrass:	1.45	0.20	0.04	0.12	0.84	251
Rukuhia peat, 2P2K:						
White clover:		0.32		0.32	1.62	
Red clover:		0.38		0.52	2.06	
Lotus sp:		0.38		0.49	1.62	
Grasses:	2.64	0.31		0.18	0.48	
Ruakura peat, 10P5K:						
White clover:	4.55	0.29	0.27	0.36	1.10	38

TABLE 17. EXP. 6. COMPARATIVE POTASSIUM CONTENTS OF FOUR SPECIES FROM TRIALS SHOWING POTASSIUM RESPONSES ON ROTORUA PUMICE SOILS
(K % in dry matter)

Trial and Date Cut		White Clover		Red Clover		*Lotus* sp.		Grasses	
		Control	+ K	Control	+ K	Control	+ K	Control	+ K
Kaharoa	Nov. 1950	1.37	1.34	—	1.08	1.00	1.22	1.90	2.16
	Mar. 1951	0.91	1.32	0.79	0.92	0.61	0.78	1.78	2.46
	Oct. 1951	1.70	2.17	1.57	1.84	1.63	2.14	2.49	3.09
Oturoa	Oct. 1950	1.26	1.48	1.04	1.68			1.67	2.37
	Mar. 1951	1.64	2.27	—	1.99			1.71	2.37
	Oct. 1951	1.51	1.94	1.59	2.36			2.49	2.90
Ngongotaha	Oct. 1949	1.06	3.15	1.08	3.62			2.02	3.33
	Mean	1.35	1.95	1.21	1.93	1.08	1.38	2.01	2.67

Average status of other nutrients in plus K plots (per cent dry matter, or p.p.m. Mn):

	N	P	Na	Mg	Ca	Mn
White clover	5.42	0.39	0.32	0.29	1.49	101
Red clover:	4.86	0.38	0.13	0.40	1.75	112
Lotus sp	4.95	0.42	0.35	0.33	1.17	145
Grasses:	3.42	0.38	0.32	0.22	0.58	210

TABLE 18. MEAN CONTENTS AND RANGE OF VALUES OF POTASSIUM IN WHITE CLOVER AND GRASSES GROWING IN ASSOCIATION
(K % in dry matter)

	No. of Trials	No. of Samples	White Clover		Grasses	
			Control	+ K	Control	+ K
Trials showing responses to applied potassium	12	20	1.52 (0.62–2.82)	1.97 (1.32–3.18)	2.09 (1.54–3.19)	2.64 (1.89–3.83)
No responses	12	17	3.10 (1.80–4.50)		3.37 (2.21–4.62)	

Average status of other nutrients in plus K plots in trials showing responses (per cent dry matter, or p.p.m. Mn):

	N	P	Na	Mg	Ca	Mn
White clover:	4.98	0.35	0.41	0.27	1.42	86
Grasses:	3.40	0.36	0.40	0.20	0.58	162

Results. The data presented in the tables indicate a rather ill-defined threshold value between 1.5 and 2.2% K, with a mean value of approximately 1.8% K for whole leaves, including immature growth, of white and red clovers at grazing height. The absence of significant responses in the red clover in Exp. 2 appears to be anomalous, especially as visual deficiency symptoms were evident in the PN plots.

TABLE 19. MEAN POTASSIUM CONTENTS OF SELECTED LEAVES SHOWING SYMPTOMS, COMPARED WITH WHOLE LEAF GROWTH FROM DEFICIENT AND HEALTHY PLANTS

	Potassium-deficient Soils								Non-responsive Soils	
	Selected Leaves		Whole Leaf						Whole Leaf	
	Controls or Insufficient K						Adequate K		Healthy Controls	
	(a) Leaves with Symptoms		(b) Mixed Sample Responses Significant		(c) Mostly No Symptoms Responses Non-significant		(d) No Symptoms Apparently Healthy		(e) No Symptoms No Responses	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
White clover	0.29–0.87	0.47 (28)	0.62–2.82	1.27 (82)	0.47–3.38	2.07 (42)	1.35–3.93	3.02 (80)	1.80–4.50	3.42 (26)
Red clover	0.28–0.64	0.43 (12)	0.51–2.58	1.30 (40)	0.48–1.55	0.91 (24)	1.49–3.86	2.80 (42)		
Sub. clover	0.37–0.47	0.40 (8)	0.46–0.76	0.61 (8)	1.25–1.71	1.48 (4)	2.20–3.08	2.49 (8)	2.63–3.48	3.18 (4)
Suckling clover	0.39–0.43	0.41 (2)	0.57–1.53	0.89 (5)			1.44–2.31	1.79 (3)		
Lotus sp.		0.33 (1)	0.50–1.73	1.02 (10)			1.92–3.57	2.82 (7)		
Lucerne, flowering			0.45–1.29	0.81 (10)	1.42–1.68	1.55 (4)	1.70–2.31	2.05 (6)		
,, , top 6″	0.86–1.34*	1.09 (4)					2.01–3.49	2.88 (6)		
,, , leaves only		0.44 (1)						1.79 (1)		
Ryegrass		0.25 (1)	0.43–1.36	0.84 (37)	0.83–2.38	1.47 (26)	1.57–4.92	3.40 (64)	2.86–5.01	4.00 (26)
Cocksfoot			0.77–1.14	0.91 (6)			3.85–4.71	4.24 (6)		4.29 (1)
Yorkshire fog			0.96–1.88	1.23 (6)			4.40–5.13	4.66 (6)		
Sweet vernal†					0.83–1.33	1.15 (12)	2.25–4.47	3.38 (12)		
Paspalum†					0.28–0.53	0.43 (12)	2.24–3.36	2.71 (11)	2.60–3.34	3.03 (6)

Number of samples in brackets. In the suckling clover, *Lotus* species, lucerne, and red clover to a less extent, the sample included stem tissue.

* Whole top 6 in., only a portion of the total weight analysed being leaves showing deficiency symptoms.

† No evidence of potassium deficiency in these two plant types in spite of marked responses in other plants in the same plots.

The critical level may be somewhat lower in subterranean clover, as is also suggested by Rossiter's data (1947, 1955). Gammon's (1953) work with pot cultures indicated a critical level in white clover of 2.0% K (average K content in the herbage of the last cutting preceding a cutting that indicated 20% decrease in yield relative to the best treatment), while Hood *et al.* (1956) reported a critical value (Macy) of 40 m.e. per 100 g (approx. 1.6% K). Ulrich (1946), on the other hand, found values of only 0.8 to 0.9% for mature leaves of Ladino white clover grown in pots.

For perennial ryegrass a critical requirement of 1.3 to 2.0% K is indicated from Exp. 2, and a mean value of about 1.6% K. Most of the significant responses were obtained in the early summer during periods of maximum growth. The absence of significant responses at other times was apparently not due to nitrogen deficiency, but various factors may have contributed, such as soil heterogeneity, rust attack, dry weather, dormancy, or failure of some plants to recover from trimming. There was no increased uptake of potassium during the periods of restricted growth in the summer and autumn. Consequently samples taken at such times when there can be no clear correlations between yield and nutrient levels, must be disregarded in the determination of critical levels. Italian ryegrass in 1953-54 showed smaller responses, significant only in the limed plots. Both cocksfoot and Yorkshire fog showed significant responses in the 1954-55 season, but neither paspalum nor sweet vernal showed any indication of responses in the same season. Potassium uptake by the paspalum from the control plots was very low (less than 0.5 to 0.6%) confirming Gammon's (1953) evidence that this genus has a very low internal potassium requirement.

In the case of clovers, which supply their own nitrogen needs, the responses have been due to potassium nutrient only. In Exp. 2 nitrogen was added, but in those experiments where grasses were dissected out from a mixture of legumes and grasses, the potassium responses could be due to both direct responses from the fertiliser and indirect nitrogen responses through the legume. Consequently data for grasses from potassium experiments with mixed pastures are of limited value for establishing critical levels, especially when the nitrogen status of the grasses is rather low. Results in Exp. 4 to 6, are, however, consistent with a critical requirement for grasses (mainly perennial ryegrass), at grazing height, of 1.5 to 1.8% K. Gammon's (1953) work in Florida indicates that in low-producing grasses the critical level may be well below 1% K in some species, but nearer 2% in a high-producing grass (*Digitaria decumbens*). Further work is needed to define the critical requirements of pasture grasses under New Zealand conditions. The indications are that cocksfoot and Yorkshire fog may have similar needs to ryegrass whereas sweet vernal and especially paspalum have much lower needs.

Multiple deficiencies and critical levels. Ulrich (1946) concluded that in estimating the critical nutrient level of a plant only the nutrient concentrations from plants definitely known to be deficient in the nutrient studied should be compared with the corresponding yields. Positive correlations of yields with potassium concentrations were obtained when potassium was limiting yields, while equally significant negative correlations with potassium concentration were found when phosphorus was limiting yields. As far as possible, therefore, samples have been taken in the present work from plants believed to be adequately supplied with all nutrients, other than potassium in deficient plants.

At certain samplings, however, yields may have been reduced through some deficiency of phosphorus and magnesium, and sometimes of nitrogen in the case of the grasses, in spite of the provision of ammonium sulphate and very heavy dressings of superphosphate. The effect of a second, but initially less acute limiting nutrient factor on the shape of the curve, and consequently the apparent critical concentration, requires careful consideration. If phosphorus, for example, is in short supply, the maximum possible yield from potassium additions will not be attained because of the development of phosphorus deficiency. At the lowest levels of potassium supply, there will normally be adequate phosphorus for the small amount of growth made by the potassium-deficient plants and consequently the lower part of the curve will not be affected. As growth increases with each increment of added potassium, phosphorus deficiency develops and the two curves diverge. This must result in a shift of the upper points of the graph downwards and to the right. It is uncertain, however, whether this in itself will necessarily also result in a shift to the right of the point where the transition curve levels out (Macy's critical percentage). If any of the response to potassium chloride should be due to indirect effects and not to potassium nutrient, this will invalidate the correlations and will result in fictitiously high apparent critical levels.

Seasonal variation in critical levels. In those trials reported in this paper, where yield measurements or estimations were made throughout the year, it was found that responses were at a minimum during periods of restricted growth in the autumn (hot, sunny, and dry weather) and winter (cold, cloudy and wet). Under favourable environmental conditions, deficiency of another element normally results in some increase of potassium concentration in plant tissues. The evidence from the present work is that climatic effects limiting growth in the autumn, on the other hand, do not cause any such build-up. The evidence for clovers at grazing height, especially from Exp. 2 and 4, suggests not only lower concentrations in the tissues during late summer and autumn but also lower apparent critical requirements over this period. During the winter and early spring there was some recovery of potassium concentrations to normal in Exp. 4, but responses were at a minimum or not detectable. Data from plants growing under unfavourable climatic conditions are clearly unsuitable for establishment of critical concentrations, but in view of the trend of results it is concluded that analytical levels for such samples have some diagnostic value for prediction of probable responses during more favourable climatic conditions.

It is unlikely that moisture is the main factor responsible for the lower apparent critical requirements during autumn, as Ulrich's (1946) work shows that similar seasonal effects can occur with Ladino white clover even under the controlled-moisture conditions of pot experimentation. When yields in his Table 6 are plotted against K levels (Tables 11 and 12) higher "critical" values are indicated for June and July cuts (summer) than for August and September (autumn). This effect may be due to "carbohydrate dilution". In hot, sunny, and dry weather, carbohydrate and lignin formation will be at a maximum and the resulting "dilution" effect may explain the lower critical internal needs indicated from autumn cuts. In field trials, as in the present work, moisture deficit and high temperature are likely to accentuate this effect, as restriction of growth means longer periods of growth between grazings or cuttings and consequently greater *maturity* of tissues analysed. Still greater maturity effects can be expected with plants sampled at the hay stage. Knowles and Watkin (1931) working on the assimilation and translocation of plant nutrients in wheat during growth, showed that potassium assimilation practically ceases by ear emergence and, later, appreciable potassium may even be lost. Chambers (1953) has published similar data. Since carbohydrate synthesis continues after ear emergence, this evidence implies a continued drop in internal potassium needs as the plant matures.

Much of the published evidence on critical levels in legumes and grasses is for hay cuts. The tentative level of 1.8% K for white and red clovers at grazing height, indicated in this work, is consistent with the findings, for example of Jackson *et al.* (1948), who set a minimum level of 1.25% K and an optimum range of 1.25 to 2.0% K for red clover and lucerne at the hay stage.

A possible complicating factor in the present trials is winter frost in Exp. 3 with white clover and in Exp. 4 to 6 with permanent pastures grown through the winter with temperatures down to 16° to 22°F (minus 6° to 9°C). In America it is claimed that a higher minimum potassium content is needed for optimum winter survival of lucerne than for first-year production in districts subject to severe frosts.

Effect of tissue analysed on critical levels. It is apparent from the evidence on relative levels of potassium in different tissues that the minimum requirement for near-maximum growth will also depend on the tissue analysed, and will be higher for petioles than leaflets in legumes and higher in stem tissues than in leaves in immature growth of red clover and lucerne. The proposed critical level of 1.8% K in whole leaves of white clover corresponds to a level of approximately 1.6% K in leaflets and 2.1% K in petioles. It is clear also from Table 19 that the critical or no-response level in legumes in the whole foliage sampled is higher than the level in selected tissues showing clearly defined symptoms. Figures in column (a) were for selected leaves (leaflet plus petiole) showing clear deficiency symptoms, whereas the values in column (b) and those in the main tables were for the whole above-ground growth (excluding dead leaves and flowers) from about 1 in. above ground level. The latter, therefore, included young as well as mature leaves and part of the difference in analytical levels is undoubtedly due to the fact that the samples were not strictly comparable in stage of growth. If clover samples used had been confined to mature leaves, a lower critical level would certainly have been indicated. This will be a factor contributing to the relatively low critical levels of only 0.8% for petioles and 0.9% for leaf blades found by Ulrich (1946) for Ladino white clover grown in pots and cut at approximately monthly intervals. In sampling for analysis, Ulrich selected only mature leaves, discarding obviously immature or senescent leaves.

It is generally conceded that youngest mature leaves are best for diagnosis of potassium deficiency, but the selection of such tissues is often impracticable in investigations of problems of nutrition of pastures under grazing. Besides being more convenient to collect, the whole of the top growth from about 1 in. above ground level allows of the utilisation of samples cut for yield measurements.

Ion substitution and critical levels. Lehr's (1951) work indicates that some grasses, including ryegrass, will respond to sodium especially when potassium is in sub-optimal supply. This partial substitution of sodium for potassium may have affected the apparent critical needs of ryegrass, as there was considerable variability in the exchangeable sodium and plant sodium uptake in the several experiments.

Effect of soil heterogeneity on critical levels. Considerable variability in available potassium contents of individual soil cores has been reported by many workers, for example Hemingway (1955). Ferrari and Vermeulen (1955) have also stressed the great variability in potassium status of soils from point to point in a field. They quote data of Schuffelen *et al.* which show nearly five-fold variability within an area of 1 sq. metre and of van der Paauw which demonstrate considerable fertility gradients over a distance of only 20 metres. In such cases a representative plant sample from a field trial, as pointed out by Ulrich (1948), may constitute merely a weighted mean of samples from plants ranging in nutrient status from severely deficient to luxury levels. Apparent "critical levels" derived from such field data may differ greatly from criteria derived from analysis of material from more uniform plots (Ulrich 1943), and especially from results of pot experiments where nearly perfect admixture can be assumed. The effect of variability in the degree of deficiency has also been discussed by Thomas (1937). The effect of a mosaic pattern can best be appreciated by considering a hypothetical case of a potassium-deficient pasture. If half the plants are acutely deficient and test 0.5% K, and half have adequate potassium and test 2.0% K, then if the yield of the deficient plants is only half that of the healthy, a representative sample, as obtained by a mowing cut, would contain 1.5% K. Top-dressing could double the yields of the deficient plants, giving a $33\frac{1}{3}$% overall increase in yield apparently associated with a test of 1.5% K, when the real facts are a 100% yield increase associated with a 0.5% test, plus a nil increase associated with a 2.0% test. Soil heterogeneity must therefore result in higher apparent values for the critical requirement when determined from field trials.

As potassium is concentrated in urine patches, this effect is likely to be more serious with plots on land grazed by stock, though Barker and Steyn (1956) found appreciable variability even on virgin soils. The effect of the grazing animal in producing or accentuating soil heterogeneity can be seen from the calculated distribution of cow urine, using the following data and assuming random distribution: Number of urinations per day 10 (Hancock 1950), less 10% to allow for losses in yards etc.; average volume of each urination 1600 ml (Doak 1952); number of cows per acre (including replacements) 0.75; area covered per urination 650 sq. inches (Doak, *loc. cit.*). In 10 years, 8% of the area will receive no urine, 20% one application, 25% two, 22% three, 14% four, 7% five, 3% six, and about 1% seven or more applications. As about 80% of the ingested potassium appears in the urine (Doak), this distribution pattern must result in considerable field variability in spite of the "levelling" effect of plant growth and removal or transfer by grazing.

Because of field variability, it may be necessary to use two sets of criteria or "critical" levels for grasses and clovers—one for samples obtained by means of a mower, the other for selected samples representing contrasting good and poor growth.

Critical levels and deficiency symptoms. Ulrich (1948) advocates the use of plant parts showing deficiency symptoms. He states that "the analysis of plant parts showing definite deficiency symptoms should give critical values, at least on theoretical grounds, that are subject to less variation than for samples taken without regard to their symptoms, as in routine field sampling". Some evidence in the literature implies that the critical or threshold level in relation to yield is aproximately the same as the level at which deficiency symptoms first appear. Other workers, however, have claimed that significant reductions in yield may occur in the absence of symptoms. In some cases this apparent contradiction may be in part due to over-simplification. Gammon and Blue (1953) report a decline in yield of white clover as the potassium concentration falls below 2% but "deficiency symptoms do not usually appear until the concentration is less than half that required for optimum growth". In a similar way to the present work, their value of 2% has been arrived at by analysis of the whole above-ground growth, but the concentration associated with deficiency symptoms relates specifically to foliage showing symptoms, that is to dominantly mature and senescent leaf tissue from selected plants. In the present work it is not certain how much of the gap between the apparent critical level in white clover in relation to yield and the level associated with deficiency symptoms is due to soil heterogeneity plus tissue selection.

Contrasting values can of course be expected in those instances where plant samples are taken for analysis during the growing period of a crop and nutrient levels are correlated not against yield of vegetative growth at the time of sampling, but against yields of grain, or hay, at final harvest, possibly months later, when the nutrient supply position may have radically changed. It is quite normal to find large differences between such "anticipatory" critical levels and the values associated with the appearance of deficiency symptoms (for example Nicholas 1952).

Sometimes, however, easily recognisable deficiency symptoms do not appear at all, in spite of large yield responses. Washko (1949) working with brome grass in sand culture reported that symptoms were slow in appearing and were not severe and concluded "it would appear, therefore, that the growth of brome grass might be inhibited by a lack of potassium, without outward signs of the deficiency and the use of symptoms, therefore be unreliable for the determination of potassium requirement". Bourdon *et al.* (1948) recorded that yields of clover dropped immediately with potassium deficiency but leaf symptoms did not appear for 12 years. Wallace and Bear (1949) found no symptoms of potassium deficiency in lucerne grown in a deficient soil in autumn although height of growth was reduced. In the present work, deficiency symptoms were absent or difficult to identify in the autumn and winter, but these were also the times of minimum responses. It is concluded that where deficiency symptoms are present, analysis of functioning leaves showing such symptoms should provide reliable diagnostic evidence of potassium deficiency, but the absence of symptoms does not necessarily preclude the possibility of responses from application of potassium fertilisers. This will also apply in cases of multiple deficiencies where potassium shortage is masked by associated deficiencies. Gregory (1949) has shown that if nitrogen and potassium are simultaneously and proportionately reduced the symptoms are characteristic of nitrogen deficiency and again potassium deficiency symptoms are entirely masked by phosphorus deficiency.

Extreme deficiency and nutrient levels. Steenbjerg (1945) has pointed out that under extreme deficiency conditions, the concentration of a nutrient may actually be greater than under conditions of milder deficiency. He has reported that Kristensen found such effects even with potassium, though examples were rare. In the present work no clear evidence has been noted of any abnormal inflexion in the curve relating potassium concentration in plant tissues with responses to fertilisers.

Competition between Grasses and Clovers

Though response evidence indicates that ryegrass has an internal potassium requirement similar to, or even less than that of clovers, the data for comparable samples in Tables 12 to 18 show that, in mixed pastures on the soils concerned, the grasses take up higher concentrations of potassium from the soil than the clovers growing in association. White and red clovers take up similar amounts, white averaging slightly higher than red, while the lower-demanding suckling clover and *Lotus* sp. have somewhat lower levels. On some soils, such as the Horotiu sandy loam, the grasses invariably take up more potassium than the associated clovers, though with luxury supplies of potassium the difference is usually less. On other soils the contrast is smaller. For example, on the non-responsive Te Kowhai soil type the average levels for dissected samples from 8 plots were practically identical (white clover 3.3%, ryegrass plus Yorkshire fog 3.4%). All the results in the Tables or discussed above were for paired samples each dissected from the one mixed sample of grass and clover. Less reliance can be placed on samples taken by selection in the field instead of by dissection.

In general, on potassium-deficient soils, grasses are more efficient at securing their potassium needs than associated clovers. Various workers (e.g. Blaser and Brady 1950) have reported that grasses take up more potassium than associated legumes in competition. This means that clovers are more sensitive indicators of potassium deficiency than grasses in a mixed pasture, and for diagnostic purposes are preferable to grasses or mixed herbage. On the Horotiu sandy loam, if clovers in a mixed pasture at grazing height are to take up their minimum requirement of about 1.8% K for optimum potassium nutrition, the associated ryegrass or other high-producing grass can be expected to contain about 2.3% K. In diagnostic work, therefore, if selected ryegrass or ryegrass-dominant mixed pasture at grazing height is analysed for the purpose of predicting responses in clovers to potassium fertiliser on this or similar soil types, a reference minimum level of 2 to 2.5% K is indicated. For a hay cut, a level of about 1.5 to 1.8% K should suffice.

Recent work, such as that of Gray *et al.* (1953) has shown that the lower the root cation-exchange capacity of the grass, the greater will be its tendency to take up relatively more K and less Ca than the associated clover. This implies that the lower the root cation-exchange capacity of the grass the higher the reference level will need to be to ensure that adequate potassium is available to the clover component of the pasture.

CONCLUSIONS

(1) In the trials on Horotiu sandy loam, yield responses commenced in spring, were at a maximum in summer, declined in the hot dry weather of autumn, and were at a minimum or not detectable in winter. The appearance of deficiency symptoms in white and red clovers closely followed this pattern of growth responses, which itself followed the seasonal growth cycle. Symptoms were slight in the autumn and not detectable in winter.

(2) Potassium applications on deficient soils resulted in appreciable increases in the concentration of potassium in plant tissues over the whole range from severe deficiency to adequacy, but on soils with high available potassium the changes were small or not detectable.

(3) Potassium concentrations are much higher in petioles than in leaflets of legumes well supplied with potassium. Differences are much smaller in potassium-deficient plants. In immature legumes with an erect habit, similar levels are found in petiole and stem tissues, and both are higher than in whole leaves. In legumes at the hay stage, however, stem tissues as a whole may have a lower concentration than the whole leaves, but in the top six-inches growth of such plants the potassium distribution pattern is similar to that of relatively young plants.

(4) Seasonal fall in potassium content of pasture species in the autumn may be due mainly to differences in maturity and lignification of tissues, associated with longer intervals between grazings, and to "carbohydrate dilution" due to increased synthesis of carbohydrates in hot, sunny, and dry weather.

(5) With one exception all legume leaves (leaflets plus petioles) with deficiency symptoms showed potassium levels below 0.7%.

(6) The critical level in relation to yield at the time of sampling, or minimum level for near-maximum growth, was approximately 1.8% K for whole leaves of white and red clover at grazing height. (Samples included immature growth as well as mature leaves.) For ryegrass, the value was approximately 1.6% K, and similar levels were indicated for cocksfoot and Yorkshire fog. Sweet vernal and especially paspalum appear to have much lower needs. Critical levels are lower in plants at the hay stage.

Factors possibly contributing towards the relatively high critical levels for clovers in relation to yield, compared with Ulrich's value of 0.8% to 0.9% K, and compared with critical levels in relation to appearance of deficiency symptoms, are (1) differences in tissue analysed, young as well as mature leaves being used in this work whereas Ulrich used mature leaves only, (2) soil heterogeneity, (3) other factors that may limit growth, including moisture deficit and phosphorus deficiency.

(7) In pastures on potassium-deficient soils, grasses are more efficient at securing their potassium needs than associated clovers. Clovers are therefore more sensitive indicators of potassium deficiency than associated grasses. When ryegrass-dominant pastures are analysed for the purpose of predicting responses in clovers to potassium fertilisers, higher reference minimum levels may be required than for pure clover stands.

(8) Responses in clovers to economic rates of potassium fertilisers are more likely in associations with grasses of high root cation-exchange capacity such as meadow fescue and cocksfoot than in browntop-dominant swards.

ACKNOWLEDGEMENTS

The assistance of the following officers is gratefully acknowledged: J. E. Allan and staff for spectrographic and flame-photometer determinations; W. M. Dill-Macky, H. J. Taylor, and Miss G. R. Thomas for technical assistance; H. A. Browning and T. F. Southon for field assistance; J. R. Murray and R. K. Ward for permission to use unpublished data from their field experiments; various field officers of the Extension Division for samples: Miss J. G. Miller, biometrician, for statistical analyses.

REFERENCES

BARKER, W. F.; STEYN, W. J. A. 1956: Errors in the Sampling of Soils for Chemical Analysis. *S. Afr. J. Sci. 52:* 169–74.

BELL, J. E. 1936: Potash Topdressing of Auckland Pastures. Response from Potash at Waihi. *N.Z. J. Agric. 53:* 359-65.

——————1937: Responses in Other Districts. *Ibid. 54:* 8-14.

——————1953: Soil Types and Potash Responses in Auckland Province. *Ibid. 87:* 472-3.

BLASER, R. E.; BRADY, N. C. 1950: Nutrient Competition in Plant Associations. *Agron. J. 42:* 128-35.

Bourdon, D.; Cotte, J.; Tsvetoukhine, V. 1948: Effects of Potassium Deficiency on the Growth of Cultivated Plants and the Evolution of the Exchangeable Potassium of the Soils. *C.R. Acad. Agric. Fr. 34:* 777-80. *(Soils & Fert.* 1949, *12,* 190.)

Bray, R. H.; De Turk, E. E. 1938: The Release of Potassium from Non-replacable Forms in Illinois Soils. *Proc. Soil Sci. Soc. Amer. 3:* 101-6.

Burgess, A. C.; Lynch, A. J. 1951: Potash Topdressing in North Taranaki. *N.Z. J. Agric. 82:* 165-72.

Chambers, W. E. 1953: Nutrient Composition of the Produce of Broadbalk Continuous Wheat Experiment. II. Changes Occurring During One Season's Growth. *J. agric. Sci. 43:* 479-84.

Chandler, R. F.; Peech, M.; Bradfield, R. 1946: Techniques for Predicting the Potassium and Boron Requirements of Alfalfa. I. The Influence of Muriate of Potash and Borax on Yield, Deficiency Symptoms, and Potassium Content of Plant and Soil. *Proc. Soil Sci. Soc. Amer. (1945) 10:* 141-6.

Chandler, R. F.; Peech, M.; Chang, C. W. 1945: The Release of Exchangeable and Non-exchangeable Potassium from Different Soils upon Cropping. *J. Amer. Soc. Agron. 37:* 709-21.

Doak, B. W. 1952: Some Chemical Changes in the Nitrogenous Constituents of Urine When Voided on Pasture. *J. agric. Sci. 42:* 162-71.

Ferrari, Th. J.; Vermeulen, F. H. B. 1955: Soil Heterogeneity and Soil Testing. *Neth. J. agric. Sci. 3:* 265-75.

Gammon, N. (Jr.) 1953: Sodium and Potassium Requirements of Pangola and Other Pasture Grasses. *Soil Sci. 76:* 81-90.

Gammon, N. (Jr.); Blue, W. G. 1953: The Sandy Soils of Florida Need Potash for Pastures. *Better Crops 37:* 25-6, 40-1.

Goodall, D. W.; Gregory, F. G. 1947: Chemical Composition of Plants as an Index of Their Nutritional Status. *Bur. Hort., E. Malling. Tech. Commun. 17:* 1-167.

Gray, B.; Drake, M.; Colby, W. G. 1953: Potassium Competition in Grass-Legume Associations as a Function of Root Cation Exchange Capacity. *Proc. Soil Sci. Soc. Amer. 17:* 235-9.

Gregory, F. G. 1949: The Interaction of Factors in Determination of Plant Yield. *Proc. Specialist Conf. Agric. Australia:* 114-23.

Hancock, J. 1950: Studies in Monozygotic Cattle Twins. IV. Uniformity Trials. Grazing Behaviour. *N.Z. J. Sci. Tech. A32: (4):* 22-59.

Hemingway, R. G. 1955: Soil-sampling Errors and Advisory Analyses. *J. agric. Sci. 46:* 1-7.

Hewitt, E. J. 1950: Foliar Diagnosis and Plant Nutrient Requirements. *World Crops 2:* 455-9.

Hood, J. T.; Brady, N. C.; Lathwell, D. J. 1956: The Relationship of Water Soluble and Exchangeable Potassium to Yield and Potassium Uptake by Ladino Clover. *Proc. Soil Sci. Soc. Amer. 20:* 228-31.

Jackson, M. L.; Evans, C. E.; Attoe, O. J.; Kaudy, J. C. 1948: Soil Fertility Level in Relation to Mineral and Botanical Composition of Forage. *Proc. Soil Sci. Soc. Amer. (1947) 12:* 282-8.

Jacques, W. A. 1956: Root Development in Some Common New Zealand Pasture Plants. IX. The Root Replacement Pattern in Perennial Ryegrass. *N.Z. J. Sci. Tech. A38:* 160-5.

Knowles, F.; Watkin, J. E. 1931: The Assimilation and Translocation of Plant Nutrients in Wheat During Growth. *J. agric. Sci. 21:* 612-37.

Lehr, J. J. 1951: Importance of Sodium for Plant Nutrition. V. Responses of Crops Other than Beet. *Soil Sci. 72:* 157-66.

Luebs, R. E.; Stanford, G.; Scott, A. D. 1956: Relation of Available Potassium to Soil Moisture. *Proc. Soil Sci. Soc. Amer. 20:* 45-50.

Lundegardh, H. (Trans. R. L. Mitchell) 1951: "Leaf Analysis". Hilger and Watts, London. pp. 1-176.

Macy, P. 1936: The Quantitative Mineral Nutrient Requirements of Plants. *Plant Physiol. 11:* 749-64.

Mes, M. G. 1954: Excretion (Recretion) of Phosphorus and Other Mineral Elements by Leaves under the Influence of Rain. *S. Afr. J. Sci. 50:* 167-72.

Millikan, C. R. 1953: Subterranean Clover. Symptoms of Nutritional Disorders. *J. Dep. Agric. Vic. 51:* 215-25.

Nicholas, D. J. D. 1952: The Potassium Status of Barley in Relation to Yield of Grain. *J. agric. Sci. 42:* 468-75.

Paton, D. F. 1950: Potash Responses on Pastures in Northern Tasmania. *Tas. J. Agric. 21:* 302-11.

Richards, F. J.; Templeman, W. G. 1936: Physiological Studies in Plant Nutrition. IV. Nitrogen Metabolism in Relation to Nutrient Deficiency and Age in Leaves of Barley. *Ann. Bot., Lond. 50:* 367-402.

Rossiter, R. C. 1947: The Effect of Potassium on the Growth of Subterranean Clover and Other Pasture Plants on Crawley Sand. II. Field-plot Experiments. *J. Coun. sci. industr. Res. 20:* 389-401.

————1955: Strain Reaction to Potassium Deficiency in Subterranean Clover. *Aust. J. agric. Res. 6:* 9-14.

Steenbjerg, F. 1945: Om Kemiske Planteanalyser og Deres Avondelse. *Tidsskr. Planteavl. 49:* 157-77.

Steinbergs, A. 1953: A Rapid Turbidimetric Method for the Determination of Small Amounts of Sulphur in Plant Material. *Analyst 78:* 47-53.

Taylor, C. R. 1950: Potash Deficiency in Land in Rotorua District. *N.Z. J. Agric. 84:* 21-2.

Taylor, C. R.; Bell, J. E. 1952: Potassic Fertilisers for Topdressing. *Ibid. 84:* 216.

Thomas, W. 1937: Foliar Diagnosis: Principles and Practice. *Plant Physiol. 12:* 571-600.

Thompson, F. B.; Elliott, I. L. 1950: The Development of Peat Land. *N.Z. J. Agric. 80:* 101-8.

Ulrich, A. 1943: Plant Analysis as a Diagnostic Procedure. *Soil Sci. 55:* 101-12.

————1946: Critical Phosphorus and Potassium Levels in Ladino Clover. *Proc. Soil Sci. Soc. Amer. (1945) 10:* 150-61.

————1948: Diagnostic Techniques for Soils and Crops. "Plant Analysis. Methods and Interpretation of Results". pp. 157-98. The American Potash Institute, Washington.

Wallace, A.; Bear, F. E. 1949: Influence of Potassium and Boron on Nutrient-element Balance in and Growth of Ranger Alfalfa. *Plant Physiol. 24:* 664-80.

Wallace, A.; Toth, S. J.; Bear, F. E. 1948: Further Evidence Supporting Cation Equivalent Constancy in Alfalfa. *J. Amer. Soc. Agron. 40:* 80-7.

Walsh, T.; Cullinan, S. J. 1945: The Effect of Wetting and Drying on Potash-fixation in Soils. *Emp. J. exp. Agric. 13:* 203-12.

Warner, A. O. 1953: Potash Responses on Horotiu Sandy Loam. *N.Z. J. Agric. 86:* 477.

Washko, W. W. 1949: Effect of Potassium upon the Nitrogen and Mineral Content of Brome Grass. *Agron. J. 41:* 101-3.

Woodcock, J. W. 1936: Some Aspects of Potash Manuring of Pastures. *N.Z. J. Agric. 53:* 193-9.

Woodman, H. E.; Evans, R. E.; Norman, D. B. 1933: Nutritive Value of Lucerne. I. Preliminary Studies of Yield, Composition and Nutritive Value (Season 1932). *J. agric. Sci. 23:* 419-58.

FUNGAL LEAF-SPOT DISEASES OF COCKSFOOT (*Dactylis glomerata* L.) IN THE MANAWATU

I. LEAF STREAK CAUSED BY *Scolecotrichum graminis* Fckl.

By G. C. M. LATCH* and H. T. WENHAM, Massey Agricultural College, Palmerston North.

(Received for publication, 18 November 1957)

Summary

Leaf streak of cocksfoot caused by *Scolecotrichum graminis* Fckl. is common in the Manawatu. The disease has also been found on timothy.

Symptoms, spore germination, and morphology of the causal organism have been described.

The causal fungus grows on a number of media but growth is slow and sporulation rare.

The disease is of little economic importance in New Zealand.

INTRODUCTION

Cocksfoot in the Manawatu has been observed to express a variety of necrotic disease symptoms of the foliage suggesting that a number of pathogenic organisms may be involved. Although Sprague (1950) has recorded fifteen fungi as foliage pathogens of *Dactylis glomerata* L. in North America, only one of these (*Mastigosporium rubricosum*) has been recorded on this host species in New Zealand (Brien and Dingley, 1951). In November 1955, a study was initiated to determine which fungi were in fact contributing to the symptom complex in this district. Five fungi were found to be involved, namely: *Scolecotrichum graminis* Fckl.; *Mastigosporium rubricosum* (Dearn et Barth.) Sprague; *Rhynchosporium orthosporum* Caldwell; *Selenophoma donacis* var. *stromaticola* (Bauml.) Sprague et A. G. Johnson; and *Stagonospora maculata* (G.) Sprague. In this and subsequent papers are presented the significant features of each pathogen and the disease it causes.

SYMPTOMS

Leaf streak is first apparent as small chocolate-coloured lesions each surrounded by a pale-yellow area. These soon elongate and within seven days may become up to 50 mm long and 6 mm wide. Some lesions stay at this size while others continue to elongate so that streaks 3-4 cm in length are not uncommon. The degree of infection varies from a few to many hundred lesions per leaf (Fig. 1). Where infection is severe, the streaks coalesce killing a considerable portion of the leaf. Older streaks are light brown and occupy the zones between leaf veins. The original site of infection is darker and generally located at the centre of the long streak. Dark fascicles of conidiophores arranged in regular rows along the lesions facilitate correct identification of the

---

* Present address: Plant Pathology Department, University of Wisconsin, Madison 6, Wisconsin, U.S.A.

N.Z. J. agric. Res. 1: 182–188.

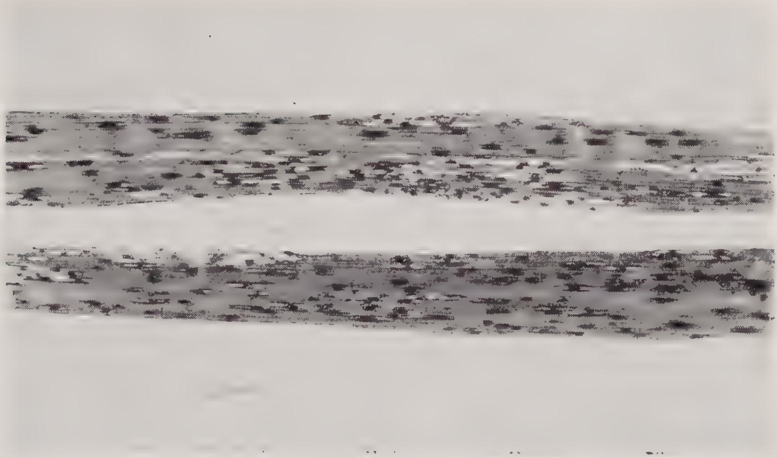


Fig. 1.—Cocksfoot leaves showing early-stage field symptoms of leaf streak.

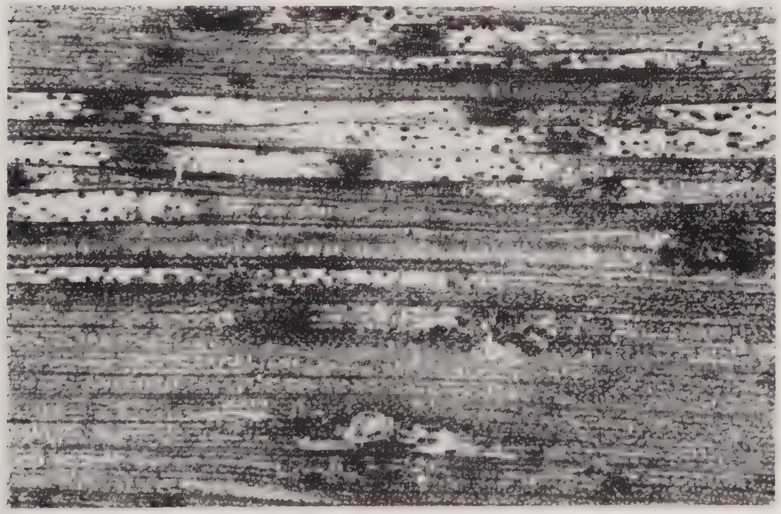


Fig. 2.—Leaf-streak lesions showing black fascicles of conidiophores (enlarged).

disease (Fig. 2). They are pinhead size, may be mistaken for pycnidia, and are formed on both leaf surfaces but generally occur on the upper surface.

Isolation and Pathogenicity

The pathogen was readily isolated by streaking a suspension of conidia across a plate of potato dextrose agar (P.D.A.) with a sterile loop. The spore suspension used was prepared by examining lesions under a binocular microscope, lightly touching fascicles of conidiophores with a sterile needle, and agitating the needle tip in a drop of sterile water.

S. graminis sporulates on culture media very sparingly. For proving pathogenicity, the inoculum used was a suspension of mycelial fragments prepared by vigorously agitating part of a mycelial thatch in a flask of sterile water. Plants to be inoculated were grown in four-inch pots of steam-sterilised soil. Leaves were rubbed between the finger and thumb to remove the "bloom", sprayed with the mycelial suspension, and then placed in a humidity cabinet. Forty-eight hours later they were removed to a glasshouse the temperature of which ranged between 15° and 22°c. As controls, four pots of plants were sprayed with sterile water but otherwise given identical treatment.

First symptoms of the disease were in evidence within 7 days. The control series remained free from disease.

HOST RANGE

The reported host range of *S. graminis* is unusually large, Sprague (1950) having recorded 141 host species in the United States. One additional host has since been recorded in the United States (Meiners 1954) and six in Alaska (Sprague 1955). Although in the course of this investigation isolations were made from foliage lesions on many common pasture species other than cocksfoot, the fungus was obtained only from *Phleum pratense* L.

TAXONOMY OF THE PATHOGEN

The fungus was first described by Fuckel on cocksfoot in 1863 and named *Scolecotrichum graminis*. Von Hoehnel (1923) considered the generic name *Scolecotrichum* Kze. invalid because of the indefinite nature of *S. virescens* Kze. the type species, and recommended the name *Passalora graminis* (Fckl.) v. Hoehn. He further claimed *Carlia recutita* (Fr.) v. Hoehn. as the perfect stage but this has never been confirmed. Horsfall (1930) suggested that the genus *Cercospora* would be more appropriate for the fungus than *Passalora* as the latter is characterised by multi-septate conidiophores while those of *Passalora graminis* (= *Scolecotrichum graminis*) are continuous. He therefore proposed the name *Cercospora graminis* (Fckl.) Horsfall. Chupp (1954) rejected this argument on the grounds that the conidia are minutely echinulate and the organism could not therefore be considered a *Cercospora*. Until von Hoehnel's work is verified by a re-investigation of *S. virescens*, neither *Scolecotrichum graminis* nor *Passalora graminis* can be definitely considered the more correct. At present both are in use, although the former is more commonly used.

MORPHOLOGY

For study of the asexual reproductive structures, temporary preparations were made using lactophenol acid fuchsin as a mountant. By examining young lesions through a binocular microscope fascicles of conidiophores were readily picked off with a needle tip and transferred to a slide. The conidiophores were dark, unbranched, and non-septate, and emerged through the stomata, particularly on the upper leaf surface. They ranged in length from 49.5 to 107.3 μ (av.

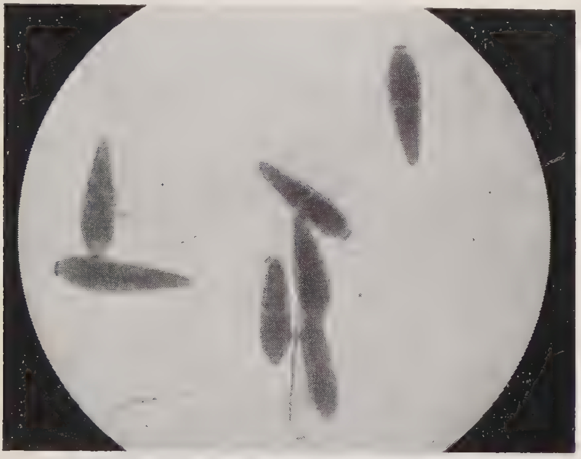


Fig. 3.—Conidia of *S. graminis* from cocksfoot, × 450.

90.8 μ). The conidia were grey-brown, cylindrical, broadly rounded at the base and tapered slightly at the tip. A well defined hilum marked the point of attachment to the conidiophores (Fig. 3). Under natural conditions the conidia were generally uniseptate, but when young lesions were subjected to periods of high humidity in petri dishes a few conidia were 2- or even 3-septate. Sprague (1950) records conidial dimensions of *Scolecotrichum graminis* as ranging from 35 to 45 μ by 8 to 10 μ. The dimensions of 219 conidia from naturally diseased cocksfoot leaves in the Manawatu ranged from 28.1 to 46.2 μ by 8.3 to 11.6 μ. Since these dimensions, also spore colour, septation, and structure are in agreement with the description by Sprague (1950), the causal fungus of the disease in New Zealand is identified as *Scolecotrichum graminis* Fckl.

GROWTH IN CULTURE

A range of media* was used to determine the colony characteristics of *S. graminis*. Inoculated plates were held at 24°c. Growth was most rapid on Difco P.D.A., cocksfoot agar, oatmeal agar, carrot agar, and prune agar, least rapid on water agar, soya-bean agar, and malt agar. At 21 days, colonies on Difco P.D.A. were 32 mm in diameter and consisted of a dark-green, slightly convoluted, raised, mycelial mat of a tough leathery nature and covered by short white aerial hyphae, particularly at the periphery (Fig. 4). All attempts at inducing production of conidia on these media by alternating light and dark conditions, exposure to sunlight, varying the temperature, and exposure to ultra-violet light were unsuccessful. A few clumps of conidia were, however, observed at 8 days on plates of cocksfoot agar (100 g cocksfoot leaves, plus 1000 ml water, plus 15 g agar agar) which had been exposed to ultra-violet light for 5 minutes at a distance of 10 cm.

---

*Difco P.D.A., cocksfoot agar, oatmeal agar, carrot agar, prune agar, cornmeal agar, water agar, soya-bean agar, malt agar.

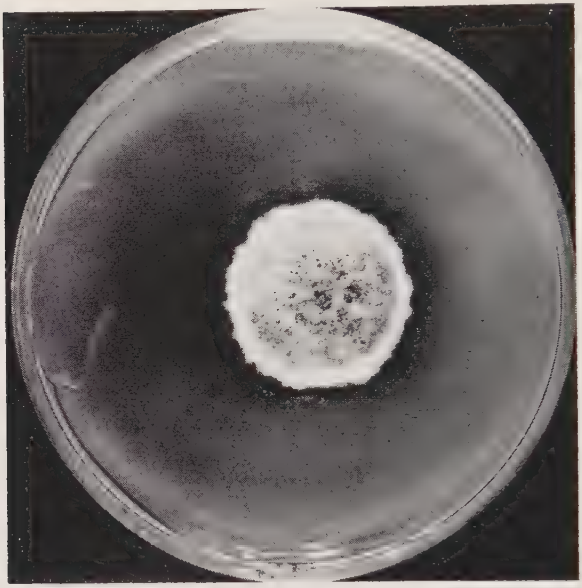


Fig. 4.—Culture of *S. graminis* held at 24°c for 21 days. Colony diameter 32 mm.

The effect of temperature on the rate of growth in culture was determined using a range of temperatures from 5° to 36°c. Cultures used in the test were grown on P.D.A., the plates each being inoculated with an 8-mm disc of mycelium from a stock culture. Measurements of colony diameter were made at the end of 9 days. Good growth was obtained between 14° and 28°c with the optimum at 24°c.

The effect of hydrogen-ion concentration on growth rate was determined by inoculating plates of buffered P.D.A. adjusted to give a series ranging in pH values from 3.0–9.0. Incubation was at 24°c. The fungus was found to be tolerant of a considerable range of pH, good growth being obtained within the range of 4.4 to 8.6, with a maximum rate at pH 5.0.

SPORE GERMINATION

The manner and rate of spore germination was studied on P.D.A. and in sterilised distilled water on glass slides held at temperatures ranging from 7° to 28°c. The slides were kept in petri-dish moist chambers and were examined at 3, 6, 10, 14, and 24 hours after beginning the tests. On P.D.A. and in water, maximum germination occurred at 24°c, being more rapid on P.D.A. A single germ tube emerged from the smaller, distal end of the spore and continued to grow for 12 to 18 hours before branching. A second germ tube from the proximal end of the spore appeared 6 to 12 hours after the initial one (Fig. 5). Lower temperatures retarded the rate of appearance of the second germ tube. At 24°c the great majority of spores had

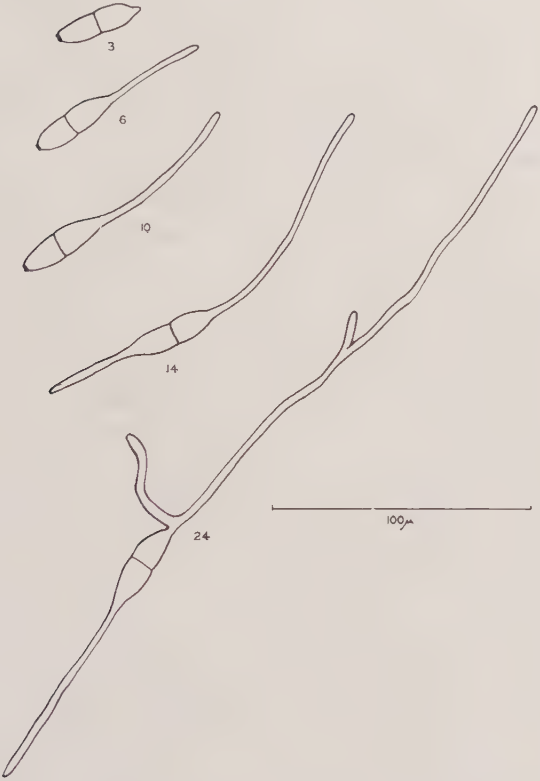


Fig. 5.—Stages in germination of a *S. graminis* conidium in distilled water at 24°C over a 24-hour period.

developed a second germ tube within 12 hours compared with approximately 50% of spores at 16°C.

SEASONAL APPEARANCE AND ECONOMIC IMPORTANCE

S. graminis causes the most important leaf spot disease of cocksfoot in the Manawatu. Observations at Whangarei, Auckland, Wellington, Christchurch, and Dunedin suggest the foregoing statement is probably true for most of New Zealand. The disease may be found at any time of the year, but is most prevalent from mid autumn until late spring. It diminishes in intensity during November and in the following two months occurs mainly on plants in sheltered locations.

Many pastures in the Manawatu with cocksfoot as a co-dominant species were inspected over two years to determine the importance of leaf streak. The disease was consistently present but in no instance considered sufficiently serious to warrant the use of control measures.

REFERENCES

BRIEN, R. M.; DINGLEY, JOAN M. 1951: *N.Z. Dep. sci. industr. Res. Bull. 101.*

CHUPP, C. 1954: "A Monograph of the Fungus Genus *Cercospora.*" C. Chupp, Ithaca, New York.

HOEHNEL, F. V. 1923: *Zbl. Bakt Abt. 2, 60:* 1–26. (Abstr. in *Rev. appl. Mycol. 3:* 102-3).

HORSFALL, J. G. 1930: *Cornell Univ. agric. Exp. Sta. Mem. 130.*

MEINERS, J. P. 1954: *Plant Dis. Reptr. 38:* 277–8.

SPRAGUE, R. 1950: "Diseases of Cereals and Grasses in North America." Ronald Press, New York.

———1955: *Plant Dis. Reptr. Suppl. 232.*

SURFACE APPLICATORS FOR THE CONTROL OF LICE AND KEDS ON SHEEP

P. L. Thomas, Scientific Officer, Animal Research Station,
Department of Agriculture, Wallaceville*

(Received for publication, 29 November 1957)

Summary

An account is given of trials using dusts, a high-pressure mist, and a low-pressure spray for the control of lice (*Damalinia ovis*) and keds (*Melophagus ovinus*) on sheep, using, principally, dieldrin as the insecticide. Three per cent dieldrin dusts, and sprays containing 0.0625% to 0.2% dieldrin gave satisfactory results, provided that a minimum of 20 mg/kg of active ingredient, distributed over the whole sheep, was deposited.

Introduction

For the past century, the dipping of sheep in insecticidal preparations to free them from external parasites has been carried out in all sheep-rearing countries of the world, and in the major areas the sheep farmers are under a legal obligation to see that their sheep are both dipped annually and kept free from parasites.

Sheep showers of various designs were introduced in Australia during the 1939-45 period and these reduced the labour of bath dipping and eliminated the risk of drowning. The process was much quicker, and up to 500 sheep could be treated per hour. However, with the dip preparations in use at that time, it was necessary to treat the sheep for four minutes in order to get the complete saturation (Duncan 1945) which was necessary for good results.

The use of a dusting machine for the control of external parasites on sheep was first favourably reported on in 1945 by Matthyse (1945) who used a 5 h.p. 18-nozzle crop duster to apply 10% cube root (containing 4.8% rotenone) in pyrophillite powder to which 2% of light motor oil was added. He advocated the method for treating "feeder" lambs infested with keds (*Melophagus ovinus*) during the autumn or winter when conditions were too severe for dipping. Over 90% control was achieved on lambs with dry greasy wool.

Denning (1949) used the same treatment on ewes immediately after shearing before lambing, in Wyoming, where pre-lambing shearing appears to be the normal practice. He reported 90 100% control of keds 14 days after treatment, overlooking it seems, the fact that ked pupae take 21–31 days to hatch.

Schwardt and Matthyse (1948) compared efficiency of dipping and dusting techniques. Dipping was said to keep a flock largely ked-free for a year, whereas the dusting gave good commercial control for several months. In the United States, "good commercial control" rather than complete control has been accepted as satisfactory and rotenone products are still in common use against external parasites.

---

* Present address: Tasman Vaccine Laboratory Ltd., Upper Hutt.

In Australia and New Zealand, where complete control has been the aim, rotenone preparations are not considered to be satisfactory—owing to their occasional failure to persist long enough after dipping to kill young keds hatching from pupae. Unless arsenic is included in the dip wash, control of the body louse, *Damalinia ovis*, is poor although again it is considered adequate in America according to Knipling (1952).

Treating sheep with insecticidal fogs was considered in Australia after a series of experiments has shown that 10% p'p' D.D.T. or 2% gamma isomer BHC gave complete control of lice on recently shorn sheep. The official report (Anon. 1953) says "it seems reasonable to consider that the results obtained are probably as good as those obtained by graziers with well recognised dips used in orthodox ways".

The fogs failed on long-woolled sheep and, as the sheep were treated in batches in enclosed spaces for periods of up to 5 minutes, severe pulmonary irritation resulted. Interest in the method has waned since 1950. However, it appears quite likely in the light of present knowledge that, if the fog were to be applied in a concentrated form to individual sheep as they passed through a race exposing them for only a few seconds, the method might be successful though such a method would appear to have no obvious advantages over dusting.

With the discovery that aldrin, dieldrin, and diazinon could diffuse down the wool fibres, greater interest has again been taken in the possibility of applying insecticides to sheep without completely soaking them in an insecticidal wash.

In 1951 and 1952, Pfadt and De Foliart (1953) in Wyoming did comparative tests of toxaphene, dieldrin, chlordane, lindane, heptachlor, rotenone, rotenone plus lethane, and rotenone plus BHC as dusts. Dieldrin at 1% was best, giving over 90% kill of keds in one application and 100% kill with two applications. In March 1955, the Registration Unit of the Pesticide Regulation Section of the United States Department of Agriculture accepted for the control of keds the use of a 1.5% dieldrin dust as a single application applied at about 1.5 oz per animal immediately after shearing.

Further work recently published by Pfadt and De Foliart (1957) showed that 1.5% dieldrin applied with a sheep-dusting machine was 100% effective against keds applied as a single treatment off shears. The results were not quite 100% when the dust was used on woolly sheep in the autumn. These authors have made no reference to the control of lice.

During the period of development with dusts, a parallel line of investigation has been carried on commercially in the United Kingdom and in Australia with a "walk-through" spray race using high-concentration dieldrin wash (up to 0.25%) applied at low pressures, principally to combat blowfly strike. In New Zealand, a high-pressure spray has been developed for the same purpose. Unfortunately, there are few published data on the performance of these spray machines apart from newspaper reports and general accounts in popular farming journals.

In 1957 there has been a concerted effort in New Zealand to determine whether these surface applicators could be as efficient for controlling lice and keds as the accepted saturation methods of application. The majority of these trials were done either at a time when blowflies were not active or in areas where blowfly strike is not important. Observations made on the control of this parasite are therefore of negligible value and are not included here.

This report covers the experiments done either solely by Departmental staff or by commercial concerns in co-operation with Departmental staff. Experiments done by commercial organisations alone are not included.

EXPERIMENTAL

Dusts

It was evident, from the American work and early commercial work in New Zealand, that the performance of dusts could be affected by the nature of the inert ingredient and the method of manufacture, therefore in the majority of dusting experiments a standard dust was used.

This dust contained ground marble with 3.0% dieldrin and 2.0% light mineral oil, both the marble and the dieldrin being ground so that 97% of the final powder was capable of passing through a 300-mesh B.S. sieve without the residue containing a disproportionately high amount of dieldrin.

Other dusts were used in later experiments using other inerts and other insecticides.

Dusting Machine

In all of the dust experiments the machine used has a dusting race with eight dust outlets, three arranged one above the other on each side, and two in the floor. Dust is fed to these through hoses from an enclosed fan revolving at 2,800 rev/min inside a housing into which dust is fed at the rate of 3½ lb per minute. With sheep running at 35-42 per minute the application rate was 1 lb to 10–12 sheep. Power was supplied in these trials by a 6 h.p. petrol engine but a tractor power take-off or electric motor could also be used.

Spray Machines

The low-pressure spray used consists of a portable 9-ft-long corrugated-iron race on a steel frame on which are attached 5 spray booms carrying a total of 20 nozzles. Wash is pumped through the spray by a small centrifugal pump powered by a petrol motor at a rate of 10 gal/min at a pressure of 15 lb/in^2.

The high-pressure spray consists of a pipe-work frame carrying 18 side jets and one overhead jet, which can be placed at the exit of an ordinary race. Wash is pumped through the spray at a pressure of 120 lb/in.^{2} which, with the jets used, gives a very fine forceful spray.

Spray Materials

In the low-pressure spray, a dieldrin emulsion was used and in the high-pressure spray a colloidal dieldrin suspension.

PRELIMINARY EXPERIMENTS

Dusting

(a) 3% dieldrin dust applied by machine to unshorn lambs.

54 unshorn Romney lambs born in October 1956 were treated by a commercial operator on 6 February 1957. These lambs carried approximately 4 inches of wool and were heavily infested with body lice (*Damalinia ovis*). The weather was warm and dry at the time of treatment.

Examinations were made at 1, 6, 14, 21, 26, and 45 days after treatment and then at monthly intervals until the end of July (5 months after treatment). The sheep were isolated from all others throughout this period.

After

1 day: No marked reduction in the number of living lice observed.

6 days: Over half of the sheep had some live lice present but not more than five were found on any one sheep. Both adults and nymphs were present.

14 days: 20% of the sheep had a few live lice present on them, all of which were nymphs.

21 days: 3 sheep had a few live nymphs present.

26 days: All sheep free from living lice.

45 days: All sheep free from living lice.

At all subsequent examinations no live lice were found.

(b) 3% dieldrin dust applied by machine to shorn lambs.

50 Romney lambs lightly infested with lice and keds were shorn on 5 February and dusted by a commercial operator on 6 February 1957. As is normal after shearing, very few keds or lice were left.

The weather at the time of treatment was warm and dry.

No live lice or keds were found 5 weeks after treatment.

Five of these lambs were then included in a concurrent dip-efficiency trial where they were exposed to reinfestation.

Lice and keds transferred to the treated sheep but all these insects were killed within seven days until 13 June 1957, when keds began to survive on one lamb. Transferring lice were all being killed until 21 July. At this stage no more infestor animals were available until the end of August, but a check made on 11 September revealed that lice were surviving on only one of the five sheep.

This result indicates that the dusting treatment was effective against keds for 3 months and against lice for at least 4 months.

In the concurrent dip-efficiency trial a group of sheep dipped in arsenical dip (0.1% As_2O_3) were not completely protected against keds, nor were they protected against reinfestation by lice after 4 months, and a BHC dip (0.004% gamma isomer) had failed against lice after four months under the same conditions of exposure to reinfestation. These strengths of arsenic and BHC are the minimum permitted in dips registered by the New Zealand Stock-Remedies Registration Board.

"Wet" Surface Applicators

(a) High-pressure spray.

Sixty-three hoggets with nine months growth of wool were treated on 17 June 1957, by the "run through" method with a high-pressure spray worked by a commercial operator using a 0.3% colloidal suspension of dieldrin. Four of these sheep were moderately infested with lice and keds and were isolated after treatment.

At the time of treatment all of the sheep were wet after very heavy rain.

At an examination five weeks after treatment all of the previously infested animals were completely free from living lice or keds.

(b) Low-pressure spray.

Sixty-five hoggets including six lice- and ked-infested animals, three of which had been rough shorn the previous day, were treated on 25 July 1957, by the run-through method with a low-pressure spray worked by a commercial technical officer using a 0.0625% dieldrin emulsion.

The manufacturers normally recommend the use of wash at twice this concentration (0.125%) for control of lice. The unshorn sheep were carrying ten months' wool.

At an examination of the sheep after five weeks, all of the animals previously infested were free from living lice and keds.

Field Trials Using "Standard" 3% Dieldrin Dust

Field trials were conducted on nineteen properties, twelve in the North Island and seven in the South Island. Some of these were done entirely by Departmental staff and in others commercial operators dusted the sheep which were examined by Departmental staff.

The trials were mainly done out of the normal dipping season when, owing to longer fleeces, higher parasite populations and adverse weather conditions, parasite control is considerably more difficult than by treatment shortly after shearing. All of these trials were done under normal farm conditions using existing yards and races.

In each trial ten or twelve sheep were examined and marked by Wallaceville staff or Livestock Instructors before treatment and were re-examined five weeks after treatment.

Results are presented in Table 1.

TABLE 1. Field Trials with 3% Dieldrin Dust Used in a Dusting Machine

Trial	Sheep		Wool	Weather	Pre-treatment		Post-treatmen (5 weeks)	
	Breed	No.			Lice	Keds	Lice	Ked
A	Romney	200	8 mths	Dry	++	—	—	—
B	Romney	114	2 in.	Wet	++	—	—	—
C	Romney	164	10 mths	Dry	+++	—	+[1]	—
D	Romney x Lincoln	80	10 mths	Damp	+++	+++	+[2]	—
E	Romney	28	8 mths	Dry	+	—	—	—
F	Romney	1,080	8 mths	Damp	+++	—	—	—
	Romney rams	11	12 mths	Damp	+	—	—	—
	Southdown rams	18	8 mths	Damp	+	—	—	—
	Romney x Southdown	48	8 mths	Damp	++	—	—	—
G	Romney	800	8 mths	Dry	+++	++	—	—
H	Corriedale	700	8 mths	Dry	++	+++	—[3]	—
I	Merino	100	8 mths	Dry	+++	+++	+	+
J	Romney	100	8 mths	Dry	++	++	—	—
K	Crossbred	900	6-18 mths	Dry	++	—	—	—
L	Romney	?	3 mths	V. wet	+++	+++	—	—
M	Romney	1,000	8 mths	Dry	++	—	+[4]	—
N	Romney	?	8 mths	Wet	+++	—	+[5]	—
O	Mixed	130	Mixed	Dry	+++	—	—	—
P	Romney ?	13	Shorn	Dry	+	—	—	—
	Romney ?	1	12 mths	Dry	++	—	—	—
Q	Romney ?	?	12 mths	Dry	—	++	—[6]	—
R	Romney	650	2-3 in.	Wet	+++	—	Not seen	
	Romney	50	3-4 in.	Wet	+++	—	—[7]	—
S	Merino	131	12 mths	Dry	—	+	—	+
	Merino	2	24 mths	Dry	—	+	—	+

[1] 1 nymph—severely affected.

[2] 1 adult louse.

[3] 6½ weeks.

[4] 1 sheep.

[5] Machine faulty.

[6] 14 days.

[7] Only half marked sheep available.

Control of both lice and keds was complete in the following trials:

A. Masterton
B. Wanganui
E. Featherston
F. Wanganui
G. Waimate
H. Waimate
J. Timaru
K. Takaka
L. Motueka
O. Palmerston North
P. Dannevirke
Q. Hastings

In the remaining seven trials, special circumstances had a bearing on the lack of success and in one other, R (Feilding), only half of the marked sheep were available for the final examination. Those seen were, however, free from infestation.

In Trial C (Dannevirke), at the post-treatment examination one nymphal louse was found to be alive on one sheep and, although it appeared affected, was observed under an illuminated lens to be capable of restricted leg movements.

In Trial D (Featherston), the sheep were damp, having been brought off hill country that morning, and their fleeces were harsh and "tippy". Five weeks after treatment one adult louse, feeding and obviously unaffected, was found on one sheep.

In Trial I (Omarama), the treated sheep were Merinos with eight months' wool, and at the post-treatment examination only four of the ten marked sheep were entirely free from infestation. A total of two lice and eleven keds were found alive on the remaining six sheep.

In Trial M (Pirinoa), one ewe was found with several living lice under the neck at the post-treatment examination. The other marked sheep, including some with much longer wool, were free from infestation. The owner observed that the operator had tended to shut the machine down for short periods when the sheep ceased to run continuously. When the flow of sheep recommenced the operator was too slow to restart the machine and the first sheep through was occasionally missed or half done.

In Trial N (Raetihi) the slide controlling the powder flow from the hopper gradually shut off owing to the vibration of the machine, and the wearing and consequent slack adjustment of the part.

A complete kill of lice was not obtained on some of the marked sheep.

This incident led to a modification being made to the design of the machine to avoid a recurrence of the fault.

In Trial S (Tekapo) the sheep treated were Merinos with twelve and twenty-four months' wool. At the post-treatment examination, of the ten sheep marked, two hoggets were found clear, one "double fleecer" had 13 keds alive on it, and a second, 3 live keds. The remainder were ewes which each had 2–8 live keds. These animals were not at any stage infested with lice.

Miscellaneous Trials Using "Non-standard" Dusts

(a) A dieldrin/pyrophillite powder prepared to standard specifications but with pyrophillite substituted for marble dust.

Four September-born Romney hoggets with heavy lice and moderate ked infestations were treated at Wallaceville, together with fifty other uninfested sheep on 1 July. These sheep were unshorn and very wet at the time of treatment.

The sheep were examined 5 weeks later and all were clear except for one apparently newly hatched louse on one sheep which appeared to be severely affected, although slight movements of the legs were obvious when it was examined with an otoscope. This louse was not removed from the sheep and its position was marked. Only dead lice could be found at this position the following day and the louse was, therefore, recorded as "severely affected and probably died in the next 24 hours".

(b) An aldrin/marble dust prepared to standard specifications but with aldrin substituted for dieldrin.

Three September-born-Romney hoggets heavily louse infested and moderately ked infested were treated under the same conditions as in 4 (a) above, but on 1 August.

Five weeks after treatment these animals were all free from live lice and keds.

(c) A diazinon (1.5%) talc dust meeting standard fineness specifications.

Three September-born Romney hoggets with heavy lice and moderate ked infestations were treated on 1 August in the same conditions as 4 (b) above.

Five weeks later these animals were all free from live lice and keds.

The drift with this dust at the time of treatment was noticeably greater than with the heavier marble and pyrophillite dusts.

Discussion

Montgomery (1954) stated that research into new insecticides was now of secondary importance in the control of livestock parasites and that the main aim was to learn how to apply insecticides.

Addison and Furmidge (1952) favoured a spray which would deposit all of its insecticide content in the fleece, and Du Toit and Fielder (1954) favoured dusts or wettable powders. All of these systems have been tested in the trials reported here.

All appear to give, with dieldrin, and possibly aldrin and diazinon, satisfactory, and in most cases complete, control of lice and keds at the concentrations used. Failures were associated with long "tippy" wool and long Merino wool where the fleece presents a hard, almost solid surface. With Merinos, it appears that treatment on short wool will be even more essential than with open-wool breeds. Other failures can be associated with human or mechanical errors which are inherent in all mechanical means of insecticide application.

From calculations made during the course of these trials, it appears that the deposition rate of 3% dieldrin dust by the machine used is approximately 40 mg/kg on an 80 lb sheep. Using 0.2% dieldrin wash, the high-pressure spray is calculated to deposit 20 mg/kg on an 80 lb sheep and the low-pressure spray using dieldrin wash at 0.125% is calculated to deposit approximately the same amount (20 mg/kg).

On the basis of these results and those of American workers who find that 1.5% dieldrin dust is adequate, it would appear that if 20 mg/kg of dieldrin can be applied to a sheep, giving complete coverage and made to stay on, it will control completely both lice and keds. Provided that the wool surface is not too hard to prevent the insecticide adhering and will allow diffusion down the fibre, and provided that the length of the wool is not so great as to cause an excessive dilution of the insecticide, it seems that both dry and wet surface applicators can be successful.

Three per cent dieldrin dust has been used in these trials as standard in order to allow a margin of excess and to assess the maximum efficiency, though it is possible that a 1.5% dust would be sufficient for control of lice and keds.

CONCLUSION

Using insecticides, such as dieldrin, which are capable of diffusing down wool fibres, it is concluded that by surface application either by dry or wet methods it is possible to control both lice or keds on sheep.

It appears necessary, however, that such applications should be made at a rate capable of depositing at least 20 mg/kg (in the case of dieldrin) on an 80 lb animal on the basis of existing information.

Application while the wool is fairly short appears advisable. Such conditions are in any case unfavourable to parasites and provide the optimum time for applying insecticides when eradication is the aim.

ACKNOWLEDGEMENTS

The help of the writer's colleagues in the Parasitology Section at Wallaceville Animal Research Station and the numerous members of the Animal Industry Division in various centres who helped with these trials is gratefully acknowledged, as is the co-operation of the following commercial enterprises: Farm Chemicals Co., Nelson; Sheep and Crop Dusters Ltd., Christchurch; A. S. Paterson and Co. Ltd., Christchurch; Summit Products Ltd., Palmerston North; Tasman Vaccine Laboratory Ltd., Upper Hutt; Ivon Watkins Ltd., New Plymouth; Cooper, McDougall and Robertson Ltd., Auckland; and Messrs. Cuddon and Stewart, Christchurch.

Further, thanks are due to the owners of the flocks of sheep that were made available for the field trials.

REFERENCES

ADDISON, C. C.; FURMIDGE, C. G. L. 1952: *J. Sci. Fd Agric. 3:* 315.

ANON. 1953 [?]: *Commonw. sci. industr. Res. Org., Melbourne, Div. Anim. Hlth. Prod. Inform. Circ. 18.*

DENNING, D. G. 1949: *J. econ. Ent. 42:* 852-3.

DUNCAN, J. E. 1945: *N.Z. J. Agric. 71:* 341-8.

DU TOIT, R.; FIELDER, D. G. H. 1954: *Onderstepoort J. vet. Res. 26:* 409-25.

KNIPLING, E. F. 1952: *U.S. Dep. Agric. Yearb. Agric. 1952:* 662-6.

MATTHYSE, J. G. 1945: *J. econ. Ent. 38:* 285-90.

MONTGOMERY, I. W. de V. 1954: *Rep. Aust. N.Z. Ass. Adv. Sci. 1954.*

PFADT, R. F.; DE FOLIART, G. R. 1953 [?]: *Univ. Wyoming exp. Sta. Bull. 23: Shell. Agric. Bull. ADB. 114.*

——— 1957: *J. econ. Ent. 50:* 190.

SCHWARDT, H. H.; MATTHYSE, J. G. 1948: *Cornell Univ. agric. Exp. Sta. Bull. 844:* 1-3.

ROOT DEVELOPMENT IN SOME COMMON NEW ZEALAND PASTURE PLANTS

X. EFFECT OF PURE SOWINGS OF SOME GRASSES AND CLOVERS ON THE STRUCTURE OF A TOKOMARU SILT LOAM

By G. S. Robinson and W. A. Jacques, Massey Agricultural College, Palmerston North

(*Received for publication, 29 October 1957*)

Summary

Pure species of three grasses and two clovers were sown on a soil of low aggregate stability. The plots were sampled at intervals for root yields and for soil aggregate analyses. Wet sieving was used to measure aggregate stability.

The grasses produced more fibrous roots than the clovers, and the soils from the grass plots had a higher aggregate stability.

The Warburg Respirometer was used to determine the rate of decomposition of dead roots in moist soil. There was a significant positive correlation between root decomposition and the resultant aggregate stability of the soils used.

The relationship between root characteristics and aggregate stability is discussed.

Introduction

In New Zealand, the assessment of the value of pasture plants has mainly hinged on their ability to produce herbage either in large amounts or at certain seasons of the year. The effects that the different pasture plants have on the physical condition of the soils in which they grow however, have not been closely studied but it seems reasonable to expect that the root form and structure would have an over-riding influence. This paper reports results from (a) two experiments which were laid down to test the effects of growing different pasture species on the structure of the top six inches of a Tokomaru silt loam and (b) a pot trial in which dead roots of the same species were added to moist soil, and the effects on structure noted after a period of incubation. Measurements of the rate of oxygen absorption were made at intervals with the Warburg Respirometer.

Soil structure improvement is largely but indirectly due to organic matter additions to the soil (Martin 1943). It has been well established that microbial and fungal activity following these additions results in the production of metabolites which make the soil aggregates water-stable. This stability persists only so long as the metabolites themselves remain undecomposed, so that a long-term improvement in soil structure is dependent on a continuing supply of organic matter to the soil or the presence of metabolites resistant to further decomposition. Pasture plants with their extensive and ever-changing root systems have been regarded as very efficient structure improvers (Jacks 1944). Baver (1956) considers, however, that, in addition to the quantitative effect of root

N.Z. J. agric. Res. 1: 199–216.

organic-matter, growing roots, in their ramification through the soil, produce localised dehydration which brings about shrinkage and the formation of fracture surfaces. Keen (1951) has suggested that the shedding of root caps, the decay of root hairs and exudations from living roots may provide the initial organic matter supply for the stabilisation of the granules formed. It is possible too, that some improvement in soil structure may be due to the actual physical binding of root hairs and of living and partially decayed roots (Russell 1950).

There is fairly general agreement amongst investigators that grasses are superior to clovers and other legumes in improving soil structure (Jacks 1944), although Ward (1949) found legumes superior to prairie grasses over three growing seasons.

Several attempts have been made to measure the effectiveness of different pasture species as soil-structure improvers and to relate the improvement to root characteristics. Stevenson and White (1941) considered that plants with tough roots were more efficient than those with slender, easily-decomposed roots. Pavlychenko (1942) suggested the "soil-binding equivalent" as a measure of the value of a grass in improving soil structure. This is the product of the length in centimetres of the roots per cubic centimetre of soil and their tensile strength. Although other investigators have shown significant differences between species (McHenry and Newell 1947), no root characteristic has been found that is directly related to this.

THE EXPERIMENTS

The experimental procedure and results are given separately for each of the following experiments:

Pure species plots sown in 1952

Pure species plots sown in 1953

Pot trial using soil to which dead roots of pure species were added.

The soil used in each experiment was a Tokomaru silt loam (N.Z. Soil Bureau Bull. n.s.5, 1954).

Pure Species Plots Sown in 1952

Experimental Procedure

The plots used in this study were sown on the Crop Demonstration Area at Massey Agricultural College. Mechanical analysis of the top six inches of this soil at the commencement of the trial showed the following percentage composition:—

Coarse sand	2.6
Fine sand	50.2
Silt	22.3
Clay	22.4
Loss by solution	0.7
Loss by ignition	7.9

The area had been cropped in the previous three years as follows:—

1949–50 Kale (inter-row cultivated)

1950–51 Kale (inter-row cultivated)

1951–52 Maize (in 14 in. rows and not inter-cultivated)

In consequence of this cropping, the soil was showing signs of a deteriorating structure, but not sufficient to give trouble in seed-bed preparation or in plant establishment.

The trial was sown on 1 April 1952, and the following were the six treatments used:

Perennial ryegrass (*Lolium perenne*)
Cocksfoot (*Dactylis glomerata*)
Chewings fescue (*Festuca ruba* var. *fallax*)
White Clover (*Trifolium repens*)
Red Clover (*T. pratense*)
Bare ground

The experiment was laid out in 10 randomised blocks each containing plots of all the treatments. The individual plots occupied an area of 10 ft by 3 ft.

The seed used was obtained from the Grasslands Division, New Zealand Department of Scientific and Industrial Research, and was of certified pedigree origin except for the Chewings fescue which was from a high-grade commercial line. The seed was sown at the rate of 60 lb per acre for the grasses and 12 lb per acre for the clovers, this being heavy enough to give quick ground cover. The area was kept clean in the early stages by hand weeding. Ample artificial fertilisers in the form of carbonate of lime, serpentine superphosphate, nitro-chalk, and sulphate of potash were applied to all plots in spring and autumn. To avoid the complicating effects of trampling and of irregular return of dung and urine, the plots were not grazed, but were defoliated with a mowing machine when growth had reached 10 12 inches in height on the majority of the treatments and a stubble of 4 inches was left. Green weights were recorded and duplicate sub-samples were taken for dry matter determinations; the method employed was that approved by the N.Z. Institute of Chemistry for plant tissues low in soluble carbohydrate (Melville 1947).

Soil blocks to be used for root studies were taken with the sampler described by Jacques (1937) and also on some occasions with the sampler shown in Fig. 1. They were brought to the laboratory and the procedure for obtaining root material closely followed the methods used by Jacques (1945). Each 2-inch layer was washed separately, and, when freed from soil and extraneous organic matter, was superficially dried between blotting paper, placed in a weighing bottle and the dry-matter determined by heating to 100°c for 16 hours. After cooling in a desiccator, the weight was recorded to the nearest milligramme. Sampling took place in April and December 1953 (i.e. 12 and 20 months after sowing the

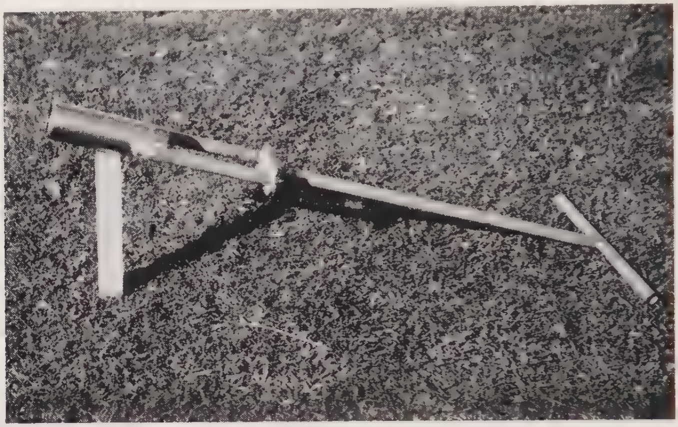


Fig. 1.—Sampler used for obtaining samples for soil-aggregate analysis.

seed). The April sampling was to a depth of 6 inches, while in December, only the 2–4 inch layer was used.

Samples for aggregate stability determinations were taken with the soil sampler illustrated in Fig. 1. The cutting edge was 2 inches in diameter and approximately 0.1 inch less than the diameter of the cylinder of the sampler. Soil samples were therefore subjected to the minimum of pressure, and damage to the structure in the sampling process was consequently minimised. The plots were sampled 4, 8, 12, and 20 months after sowing, to a depth of 4 inches on the first two occasions, and to 6 inches on the last two.

The actual sampling was done several days after soaking rain so that the soil was near field capacity. The samples were then air-dried and broken down to pass through a $\frac{1}{4}$-inch sieve. This material was dry-sieved on a 3 mm round-holed screen, and the soil retained—referred to later as 3–6 mm air-dry soil—was used for wet-sieving analyses, the method being similar to that used by Russell and Feng (1947). A 30 g sample was agitated for 5 minutes on an eight-inch 1.676 mm sieve after a pre-soaking period of 3 minutes. The sieving apparatus raised and lowered the sieve through a distance of $1\frac{1}{2}$ inches 32 times per minute. The soil left on the sieve was then oven dried at 100°c for 16 hours and weighed when cool.

Yields of Tops and Roots

Top Growth. Table 1 shows the dry matter yields of leaf and stem from each of the treatments. The yield from the white clover has been adversely affected by using a cutting height of 4 inches. The prostrate habit of growth of this species resulted in much of the leafage lodging below the mower knife.

Root Growth. Tables 2 and 3 show the relative yields of roots of the sown treatments 12 and 20 months after sowing.

TABLE 1. HERBAGE YIELDS OVER THE EXPERIMENTAL PERIOD
(lb dry matter per acre)

Period	Perennial ryegrass	Cocksfoot	Chewings fescue	White clover	Red clover
1.4.52 to 4.5.53	5830	8706	5226	2496	10453
4.5.53 to 31.12.53	5801	8915	5488	3926	8622
Total (21 months)	11631	17621	10714	6402	19075

TABLE 2. YIELDS OF DRY MATTER OF ROOT TISSUE 12 MONTHS AFTER SOWING
(lb D.M. per acre)

Treatment	Soil Layer (inches)			
	0–2	2–4	4–6	0–6
Chewings fescue	2800	822	630	4252
Perennial ryegrass	2250	686	480	3416
Cocksfoot	1939	555	408	2902
White clover	1090	323	229	1642
Red clover (total)	2880	999	463	4342
Red clover (fibrous roots only)	801	363	311	1475
Sig. diff. 5%		329		500
1%		438		670
Coefficient of variation		34.8%		

TABLE 3. YIELD OF DRY MATTER OF ROOT TISSUE 20 MONTHS AFTER SOWING
(lb D.M. per acre)

Treatment	Soil Layer (inches) 2–4
Chewings fescue	1284
Perennial ryegrass	809
Cocksfoot	609
White clover	437
Red clover (total)	1369
Red clover (fibrous roots only)	517
Sig. diff. 5%	199
1%	268
Coefficient of variation	35.2%

After twelve months' growth Chewings fescue and red clover gave a significantly higher yield at the 1% level than any of the other treatments. At the 5% level, ryegrass was better than cocksfoot, while white clover gave a significantly lower yield than all other treatments at the 1% level. It was noted, however, that over 66% of the total root-yield of red clover was in the main tap root; this left the weight of fibrous roots less than that of white clover.

After twenty months' growth, Chewings fescue and red clover again gave a significantly higher yield than the others at the 1% level while ryegrass was superior to white clover at the same level; 62.2% of the red clover roots were tap roots.

Aggregate Analysis

Effect of Species Sown. The means of the percentage aggregates in the 0–4 inch depth retained on a 1.676 mm sieve 4 and 8 months after sowing are given in Table 4.

TABLE 4. PERCENTAGE OF STABLE AGGREGATES 4 AND 8 MONTHS AFTER SOWING

Treatment	Sampling Period	
	4 months after sowing	8 months after sowing
Perennial ryegrass	64.1	77.0
Chewings fescue	62.5	79.6
Cocksfoot	60.5	77.8
White clover	60.6	74.7
Red clover	60.4	72.7
Bare ground	56.6	60.8
Sig. diff. 5%	2.1	2.6
1%	2.8	3.5
Coefficient of variation	7.6%	5.7%

The 4-month means were obtained from four determinations from each of the ten replicates. Soil from the ryegrass plots had a higher aggregate stability than that of all other treatments, except Chewings fescue. This was significant at the 1% level of probability. Soil from all five plots sown with grasses and clovers had greater aggregation at the 1% level than that of the bare-ground sample.

The 8-month means were obtained from two determinations from each of the ten replicates. At this stage Chewings-fescue soils had a better aggregate stability than those of white clover, red clover, and bare ground, all at the 1% level of probability.

The means of the percentage aggregates in the 0–6 inch depth retained on a 1.676 mm sieve 12 and 20 months after sowing were transformed to angles for the analysis of variance. The decoded data are given in Table 5.

TABLE 5. PERCENTAGE OF STABLE AGGREGATES 12 AND 20 MONTHS AFTER SOWING

Treatment		Sampling Period	
		12 months after sowing	20 months after sowing
Perennial ryegrass		85.6	90.0
Chewings fescue		85.5	90.1
Cocksfoot		85.8	89.5
White clover		80.8	86.3
Red clover		75.0	83.3
Bare ground		69.4	78.8
Sig. diff.	5%	3.0	2.5
	1%	4.0	3.3
Coefficient of variation		6.16%	5.93%

The 12-month means were obtained from two determinations from each of the ten replicates. There were no detectable differences between the three grasses but with all other treatment comparisons the differences were significant at the 1% level.

The 20-month means were obtained from one determination from each of ten replicates. The differences were very similar to those at the earlier date except that the significance was at the 5% level between cocksfoot and white clover and between white clover and red clover.

Effect of Depth. The samples taken 12 and 20 months after sowing were to a depth of 6 inches and were analysed in three 2-inch sections. The result of these analyses is shown in Table 6.

TABLE 6. PERCENTAGE OF STABLE AGGREGATES OVER ALL TREATMENTS AT DIFFERENT DEPTHS

Depth (in.)	Sampling Period	
	12 months after sowing	20 months after sowing
0–2	83.5	90.5
2–4	79.7	86.3
4–6	77.9	82.2
Sig. diff. 5%	1.1	1.2
1%	1.6	1.5

Differences significant at the 1% level of probability occurred between all depths at 12 and 20 months after sowing. In each sample there was a highly significant interaction between treatment and depth, and this can be accounted for by the clovers having a higher-than-expected stability in the upper soil layers in comparison with that in the 4–6 inch layer. This is illustrated in Table 7.

TABLE 7. PERCENTAGE AGGREGATE STABILITY FOR SPECIES AND SOIL DEPTHS FOR THE 20 MONTH SAMPLE

Treatment	Soil Layer (inches)		
	0–2	2–4	4–6
Perennial ryegrass	92.4 (105.5)	90.0 (102.7)	87.6 (100)
Cocksfoot	92.4 (107.1)	89.7 (104.0)	86.3 (100)
Chewings fescue	91.9 (103.9)	90.0 (101.8)	88.4 (100)
White clover	91.9 (113.6)	86.2 (106.5)	80.9 (100)
Red clover	91.0 (121.4)	83.9 (112.0)	75.0 (100)
Bare ground	83.2 (110.8)	78.0 (103.9)	75.1 (100)

(Figures in parenthesis show relative stability when the 4–6 inch layer for each species is taken as 100)

Effect of Age of Plots. A gradual increase in aggregate stability on all plots took place during the progress of the trial. Analysis of this trend was not made because other factors apart from age such as variations in temperature and moisture conditions both at sampling time and during air drying of the samples could be partly responsible for the differences recorded.

Pure Species Plots Sown in 1953

Experimental Procedure

These plots were established adjacent to those sown the previous year, with the primary objective of using them to gain some information on the early root-development of the species concerned, as this had not been obtained in the previous year.

The area had been cropped for the previous four years as follows:

1949–50	Barley
1950–51	Swedes (intercultivated)
1951–52	Kale (intercultivated)
1952–53	Rape (intercultivated)

The plots were sown on 14 April 1953, the soil and climatic conditions being very similar to those of the previous year. Because they were for temporary use only they were made small—3 feet square—and were replicated eight times in randomised blocks. In addition to the treatments used in the previous year, an Italian ryegrass (*Lolium multiflorum*) plot was sown and a bare-ground plot was treated with the soil conditioner Krilium to comply with the manufacturer's instructions.

Methods of establishment, maintenance, and sampling were similar to those used in the previous year's plots.

Yields of Roots

Top-growth yields were not taken from these plots, but they were sampled for root yields to a depth of four inches four months after sowing. Yields are recorded in Table 8.

TABLE 8. YIELDS OF DRY MATTER OF ROOTS 4 MONTHS AFTER SOWING (lb D.M. per acre)

Treatment	Soil Layer (inches)		
	0–2	2–4	0–4
Perennial ryegrass	1208	519	1727
Cocksfoot	1045	421	1466
Chewings fescue	933	372	1305
Red clover	931	331	1262
White clover	826	275	1101
Italian ryegrass	1180	474	1654
Sig. diff. 5%	120		188
1%	161		253
Coefficient of variation	18.5%		

At the 1% level of probability, the overall yield of perennial ryegrass was significantly better than that of all other treatments except Italian ryegrass; Italian ryegrass was better than Chewings fescue, red clover, and white clover; cocksfoot was better than white clover. At the 5% level of probability Italian ryegrass was significantly better than cocksfoot, chewings fescue better than white clover, and cocksfoot better than red clover.

Aggregate Analysis

Effect of Treatments. The mean values of the percentage aggregates in the 0–4 inch depth sampled 4 months and 8 months after sowing are given in Table 9.

The 4-month means were obtained from a single determination from each of the eight replicates. At the 1% level of probability the Krilium-treated soil was significantly better than all other treatments, the sown species all better than bare ground, and Chewings fescue and Italian ryegrass better than cocksfoot. At the 5% level, Chewings fescue was better than perennial ryegrass, white clover, and red clover.

The 8-month means were also obtained from a single determination from each of the eight replicates. The positions were very similar to those at the 4-month stage, except that all had improved, and the cocksfoot soil was relatively better and perennial ryegrass was relatively poorer in stability. The following differences were all significant at the 1%

TABLE 9. PERCENTAGE OF STABLE AGGREGATES RETAINED ON A 1.676 mm SIEVE 4 AND 8 MONTHS AFTER SOWING

Treatment	Sampling Periods	
	4 months after sowing	8 months after sowing
Krilium—bare ground	72.5	88.3
Chewings fescue	48.7	72.2
Italian ryegrass	46.2	69.3
Perennial ryegrass	43.6	66.1
Cocksfoot	39.1	69.0
White clover	43.4	69.3
Red clover	42.1	69.3
Bare ground	28.6	62.7
Sig. diff. 5%	5.0	4.5
1%	6.7	6.0
Coefficient of variation	10.9%	6.3%

level of probability. Krilium-treated soil was better than all other treatments. Chewings fescue was better than perennial ryegrass and bare ground. Italian ryegrass, red clover, white clover, and cocksfoot were better than bare ground.

Effect of Depth. Eight months after sowing, the sampling for structure analysis was made to 6 inches and was analysed in three 2-inch sections. These are shown in Table 10.

TABLE 10. PERCENTAGE OF STABLE AGGREGATES AT DIFFERENT DEPTHS

Depth (in.)	Stable Aggregates
0–2	75.2
2–4	66.4
4–6	64.1
Sig. diff. 5%	1.9
1%	2.6

The differences between the top and the two lower layers were significant at the 1% level, and between the two lower layers at the 5% level.

Pot Experiment

Experimental Procedure

The rate of decomposition of the dead roots of the species under consideration was determined by mixing quantities of the oven-dried roots with moistened soil and recording at intervals the oxygen absorption rates. 3 g of root material, which had been finely ground in a hammer mill, was mixed with 200 g of 1–3 mm air-dry aggregates of soil of low aggregate stability obtained from the plot area sown in 1953, and placed in 3½-inch plastic pots. In addition to using roots from sowings that had been established for 12 months, other pots were prepared using roots from younger plants, and four comparisons between 3 g and 6 g root additions were made; 65 g of water was added to each pot to bring the soil to approximate field capacity, at which level it was maintained by covering the pots with polythene plastic covers. Duplicate series of pots were kept in an unheated glasshouse where the minimum daily temperature ranged from 42°F to 72°F and the maximum from 73°F to 103°F. Samples were taken from the pots at intervals over the first 23 days and the extent of micro-organic activity—indicating the rate of decomposition of the root material—was determined by the Warburg Respirometer using the modifications suggested by Webbley (1947) and A. D. Rovira (pers. comm.). Triplicate determinations of oxygen absorption rates per gramme of oven-dried soil were recorded over a period of 6 hours for each treatment.

The effect of the root decomposition on the structure of the soil was measured by wet-sieving samples taken from one of the duplicate series of pots 30 days after the start of the experiment, and from the other series 40 days later. The method of wet-sieving was similar to that employed with the plot soils except that the sieve size was reduced to 0.25 mm.

Root Decomposition

The changes that occurred over the incubation period of 23 days are shown in Fig. 2 and Table 11. Determinations were made on three occasions, viz. one, eight, and twenty-three days after moistening the soil.

TABLE 11. RATE OF OXYGEN ABSORPTION IN MICROLITRES PER GRAMME PER HOUR OF OVEN-DRIED SOIL (3 g dried root per pot)

Treatment	Incubation Period		
	1 day	8 days	23 days
Control	7.1	2.6	1.8
Cocksfoot	9.7	4.5	1.9
Perennial ryegrass	9.6	5.4	2.1
Chewings fescue	14.4	6.9	2.2
White clover	27.3*	8.9	2.3
Red clover	24.7*	9.9	2.3
Sig. diff. 5%	9.5	3.8	
Coefficient of variation	39.8	32.2	16.0

* Roots from young clover plants were used in this determination but in the 8 and 23 days incubation periods the roots were the same age as the grasses.

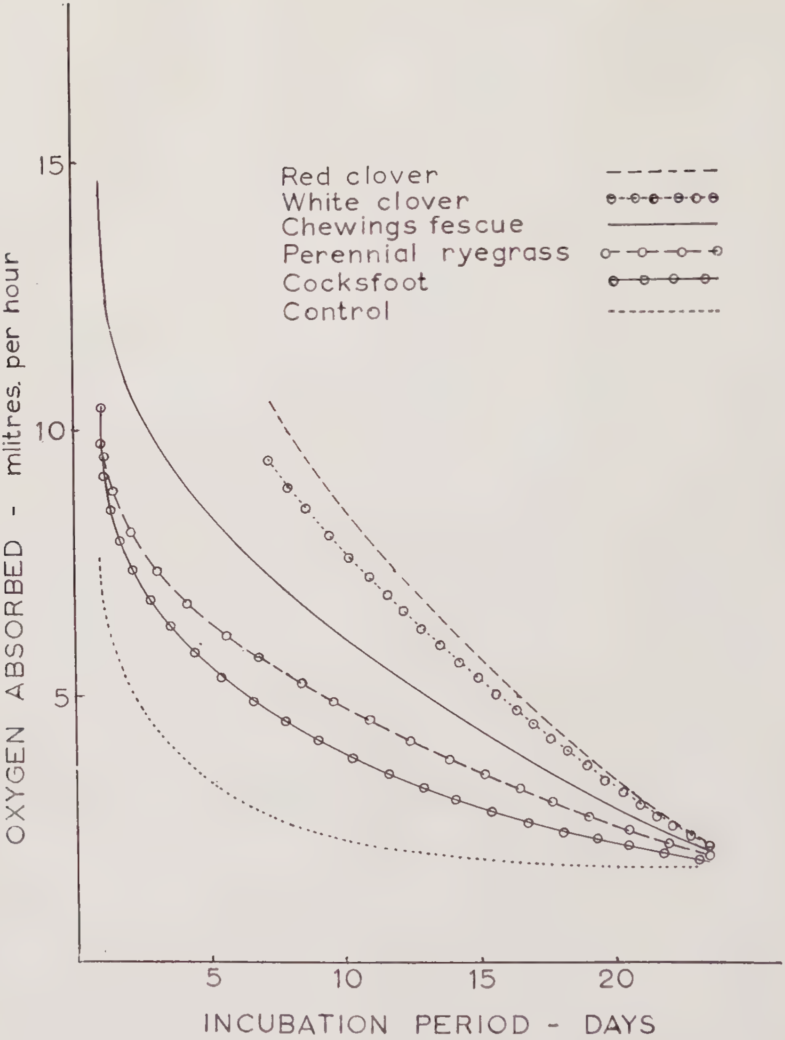


Fig. 2.—Oxygen-absorption rates of incubated soils after additions of dead grass and clover roots.

As found by Rovira (1953), rates of absorption of all treatments were high initially and tapered off as the incubation period was extended. At each determination the control soil gave the lowest oxygen-absorption rate. There were no significant differences between the grass species. The two clovers were very similar to each other in their effects with a general trend towards more rapid decomposition than the grasses. This was significant after one day's incubation where the clover roots were from 4-month-old plants in comparison with roots from 12-month-old grass plants. After 8 days incubation with roots of similar age the differences between red clover on the one hand and cocksfoot and perennial ryegrass on the other were significant as also was the difference between white clover and cocksfoot.

Aggregate Analysis

Effect of Species. Those pots to which 3 g of dried roots had been added were sampled for aggregate stability after 30 days. The percentage of oven dried soil retained on a 0.25 mm sieve is shown in Table 12.

TABLE 12. PERCENTAGE OF STABLE AGGREGATES RETAINED ON A 0.25 mm SIEVE AFTER 30 DAYS INCUBATION (3 g dried root per pot)

Treatment	% Aggregate Stability
Control	61.2
Perennial ryegrass	64.6
Chewings fescue	65.9
Cocksfoot	66.6
Red clover	67.0
White clover	71.3
Sig. diff. 5%	3.4
1%	4.6
Coefficient of variation	3.5%

At the 1% level of probability, the white clover soil was higher in aggregate stability than those of cocksfoot, Chewings fescue, perennial ryegrass, and the control; and red clover, cocksfoot, and chewings fescue were higher than the control.

TABLE 13. PERCENTAGE OF STABLE AGGREGATES RETAINED ON A 0.25 mm SIEVE AFTER 70 DAYS INCUBATION (3 g dried root per pot)

Treatment	% Aggregate Stability
Control	61.7
Cocksfoot	67.1
Perennial ryegrass	69.8
Chewings fescue	70.6
Red clover	71.8
White clover	73.8
Sig. diff. 5%	3.7
1%	4.9
Coefficient of variation	3.5%

At the 5% level of probability the white clover soil was higher than that of red clover.

The percentage of oven-dry soil retained on a 0.25 mm sieve 70 days after roots and water had been added is shown in Table 13.

At the 1% level of probability all soils to which roots had been added were higher in aggregate stability than that of control, and white clover was higher than cocksfoot.

At the 5% level of probability the white-clover soil was higher than that of perennial ryegrass.

Effect of Quantity of Roots. In the case of four of the species, both 3 g and 6 g additions of oven-dried roots were made. A comparison of the effects of this on soil structure is given in Table 14.

TABLE 14. EFFECT OF ADDING GRASS AND CLOVER ROOTS AT TWO DIFFERENT RATES ON THE PERCENTAGE STABILITY OF SOIL AGGREGATES AFTER 70 DAYS INCUBATION

Species	Weight of Root per Pot	
	3 g	6 g
Cocksfoot	67.1	76.9
Perennial ryegrass	69.8	76.1
Chewings fescue	70.6	79.2
Red clover (tap root only)	75.9	88.7
Mean	70.9	80.2

The difference between the means was significant at the 1% level of probability as also was each individual comparison.

Correlation of Root Characteristics with Soil Structure

Effect of Root Weight

With the object of looking into the relationship of root weight and soil structure, an examination of the data obtained 4, 12, and 20 months after sowing was made. From an overall inspection it was clear that the

TABLE 15. RELATIONSHIP BETWEEN ROOT WEIGHT AND SOIL AGGREGATE STABILITY (data from pure species plots of grasses and clovers)

Growth Period of Plots	Content of Sample	Correlation Coefficient
4 months	4 grasses and 2 clovers	+.514**
12 ,,	3 ,, ,, 2 ,,	+.635**
20 ,,	3 ,, ,, 2 ,,	+.613**

** Indicates significance at the 1% level of probability soil in the plot containing the least roots, i.e. bare ground, was the poorest in stable aggregates. The clover plots were next in yield of fibrous roots and the soils were significantly better in stable aggregates. The grass plots contained the most roots and these soils were the highest in aggregate stability. Correlation coefficients calculated on these were significant at the 1% level (Table 15).

Within the grasses themselves, however, the relationship between root weight and structure was not so apparent, and it was only with the samples taken four months after sowing, that the correlation coefficient was significant (Table 16).

TABLE 16. Relationship Between Root Weight and Soil Aggregate Stability (data from pure grass species plots only)

Growth Period of Plots	Content of Sample	Correlation Coefficient
4 months	4 grasses	+.416*
12 "	3 "	+.018
20 "	3 "	+.077

* Indicates significance at the 5% level of probability

The Effect of Rate of Decomposition of Dead Roots

The pot experiment shows the rate of decomposition of the grass and clover roots, measured in terms of oxygen absorption at different stages of incubation. Correlation coefficients of rate of oxygen absorption with the stability of aggregates of the soil were calculated and are presented in Table 17.

TABLE 17. Relationship between Rate of Oxygen Absorption and Subsequent Soil Aggregate Stability

	Aggregation 30 days after addition of roots (cor. coeff.)	Aggregation 70 days after addition of roots (corr. coeff.)
Rate of O_2 absorption 1 day after moisture added	+.893**	+.890**
Rate of O_2 absorption 8 days after moisture added	+.846**	+.895**
Rate of O_2 absorption 23 days after moisture added	+.759*	+.789*

** significant at 1%

* significant at 5%

DISCUSSION

In many countries both legumes and grasses have been found to be effective in improving soil structure. In some cases grasses have been superior to legumes (Jacks 1944) while in others the opposite has been the case (Ward 1949). In the pure-species plots sown in 1952 in this experiment where the method used gave a measure of stability rather than of size distribution of aggregates, the grasses were consistently better than the clovers.

This relative effect on soil structure could have resulted from several related causes:

Through the degree of root development that had taken place since sowing. It can be assumed that in the early samplings root weight is a reasonable indication of root development up to that time. Root samples taken four months after sowing in this experiment would be expected therefore to include nearly all roots that had developed up to then, and there was, in fact, a significant correlation between root weight and the aggregate stability. When the samples taken 12 and 20 months after sowing were used, the correlation was also significant but was due almost solely to the fact that the clovers gave both lower root weight and poorer structure than the grasses (Tables 15 and 16). Correlations calculated from a series of samples taken from individual pure-species plots showed no significance. The highly significant correlation of root weight with soil-aggregate stability obtained when samples from both grass and clover plots were combined in the calculation, and the lack of significance when samples were restricted to the grasses or clovers alone, indicates the care that must be taken in drawing conclusions from samples taken from a mixed pasture. Sampling sites must be in all ways comparable if satisfactory comparisons are to be made.

Through the extent to which the roots ramify through the soil. Although no measurements were made in this trial of root length, it is known that the grasses produce a more extensive root system than the clovers (Jacques 1943). This was not so apparent 4 months after sowing (Table 8), when the clover roots were only slightly coarser and little less in weight than the grasses. At later samplings (Table 2) clover ramification as implied by coarse rootlets and low root weight was much less than that of the grasses. These observations support the theory that intensity of root integration into the soil affects structure. This could be due to the formation of surfaces of fracture in soil clods, to the roots pressing the soil units together, or to excretions cementing the soil particles into granules.

Through the quantity and quality of the dead roots left in the soil. No satisfactory technique has so far been devised to determine the extent of root decay taking place, but an attempt to estimate root replacement each year in grasses and clovers was made by Jacques and Schwass (1956) with 3 grasses. Indications are that there is a considerable degree of root replacement in the common pasture species used in this experiment. That these decaying roots have an effect on soil structure is shown in the results obtained in the pot experiment. The fibrous roots of the clovers decayed more rapidly than did those of the grasses and, where similar weights of each had been added to the soil, a greater improvement in soil-aggregate stability resulted from the clovers. If, as seems likely, root-decomposition products are responsible for a substantial part of soil structure improvement, then it can be assumed that the supply of dead clover roots in the pure-species plots must be smaller than that of the grasses.

Soil structure improved over the experimental period in the bare-ground as well as the species plots. There are two possible reasons for this. One is that the original organic matter content of the soils—as indicated by a loss on ignition of 7.9%—was high enough to provide a satisfactory substrate for an appreciable biological activity and to produce sufficient metabolites to bring about an improvement in the soil structure. The second possibility is that the roots and top growth of the weeds that established on the bare ground plots between cultivations and were not removed after hoeing became incorporated into the soil and led to an improvement in its structure.

The highest soil-aggregate stabilities were recorded in the surface layers of the soil and these declined in the 2–4 in. and 4–6 in. soil layers. This no doubt is due to the greater biological activity of the top soil induced by the greater supply of residual root tissues and the better aeration. Treatments were not consistent in the relative stabilities recorded from the different soil levels. Table 7 shows that the clovers, and to a lesser extent bare ground, had a relatively high recording for the 0 2 in. soil level. Ward (1949) found where grasses and clovers were left undefoliated that "in the surface three inches of soil modifications of soil structure were more highly correlated with characteristics of the above-ground portions of the cover plants than with the properties of the roots in this soil layer". Defoliation in the present experiment was less complete for the clover plots than for the grasses. Weeds hoed on the bare-ground plots were not removed.

Although no differences were recorded in aggregate stability between the grasses in the later samplings, there was a difference in the appearance of the soils. The Chewings-fescue soil, particularly in the top 2 inches, was more finely divided than that from the perennial ryegrass, and cocksfoot soil was intermediate in this respect. Both Chewings fescue and cocksfoot produced a granular type of structure in comparison with a fragmental type present in the ryegrass soil. The clover and bare-ground soils were more like the latter. These differences in appearance were much less noticeable in the 2–4 in. layer and had almost disappeared in the 4–6 in. layer.

REFERENCES

BAVER, L. D. 1956: "Soil Physics", 3rd ed., John Wiley, N.Y.

JACQUES, W. A. 1937: *N.Z. J. Sci. Tech. 19:* 267-70.

——————1943: *Ibid. A25:* 91-117.

——————1945: *Ibid. A26:* 367-71.

JACQUES, W. A.; SCHWASS, R. H. 1956: *Ibid. A37:* 569-83.

JACKS, G. V. 1944: *Joint Publ. Commonw. Agric. Bur. 6.*

KEEN, B. A. 1951: *Proc. Spec. Conf. Plant Animal Nutrit. Australia* pp. 98-9.

MCHENRY, J. R.; NEWELL, L. C. 1947: *J. Amer. Soc. Agron. 39:* 981-93.

MARTIN, J. P. 1943: *Proc. Soil Sci. Soc. Amer. 7:* 218-22.

MELVILLE, J. 1947: *J. N.Z. Inst. Chem. 11:* 104-8.

N.Z. SOIL BUREAU 1954: *N.Z. Soil Bur. Bull. n.s. 5.*

PAVLYCHENKO, T. 1942: *Nat. Res. Counc. Canada. Bull. 1088.*

ROVIRA, A. D. 1953: *Nature, Lond. 172:* 29-30.

RUSSELL, E. J. 1950: "Soil Conditions and Plant Growth", 8th ed., Longmans Green, London.

RUSSELL, M. B.; FENG, C. L. 1947: *Soil Sci. 63:* 299-304.

STEVENSON, T. M.; WHITE, W. J. 1941: *Sci. Agric. 22:* 108-18.

WARD, H. S. 1949: *Ecol. Monogr. 19:* 145-71.

WEBLEY, D. M. 1947: *J. agric. Sci. 37:* 249-56.

THE CONTROL OF LICE (*Damalinia ovis*) AND KEDS (*Melophagus ovinus*)—A COMPARISON OF THE EFFICIENCY OF COMMON INSECTICIDES

By P. L. Thomas, Scientific Officer, Animal Research Station,
Department of Agriculture, Wallaceville*

(Received for publication, 29 November 1957)

Summary

An account is given of comparative trials in which dips containing arsenic, BHC, aldrin, and dieldrin were used. At the minimum concentrations used (0.1% as As_2O_3, 0.00125% gamma isomer, 0.00125% and 0.00125% resp.) complete kill of lice (*Damalinia ovis*) was obtained in every case, and, in addition, at least three months protection against reinfection was obtained.

Against keds (*Melophagus ovinus*) all except arsenic were efficient. Jetting with diazinon (0.0025%) and dusting with dieldrin 3% were also completely effective against both parasites.

Introduction

These experiments were planned, initially, to compare the efficiency against lice of BHC, aldrin, and dieldrin at equal concentrations, when used as emulsion dips. This was done in the first series of experiments, in 1956, and two further groups were added, one treated with a commercial arsenic/derris/sulphur dip and one treated with BHC at the strength most frequently used in commercial preparations. These latter groups were included to test the assertion frequently made by farmers in the last few years, that the older arsenical dips were superior to BHC dips for louse control.

The second series of experiments, in 1957, was planned to show the comparative efficiency of the same insecticides at the minimum strengths permitted in New Zealand. It had been hoped to include a diazinon dip in this series at the minimum strength allowed for aldrin and dieldrin, but no stable emulsion suitable for dipping was available at the time when the experiment was started. Instead, sheep were jetted to saturation and excess, giving, as far as possible, all-over coverage. In this trial an extra group was included, to which dieldrin dust was applied as a surface treatment using a horticultural hand-operated duster.

Materials

1956 Experiments

The following dipping materials were used:

(1) 20% dieldrin emulsifiable concentrate diluted to give 0.00125% in the wash.

(2) 40% aldrin emulsifiable concentrate diluted to give 0.00125% in the wash.

---

* Present address: Tasman Vaccine Laboratory Ltd., Upper Hutt.

N.Z. J. agric. Res. 1: 217–223.

(3) 5% gamma-isomer BHC emulsifiable concentrate diluted to give 0.00125% in the wash.

(4) 5% gamma-isomer BHC emulsifiable concentrate diluted to give 0.00625% in the wash.

(5) Commercial powder dip containing 0.19% soluble arsenic and 0.007% rotenone with sulphur in the wash.

1957 Experiments

The same materials were used but at the following strengths in the final washes:

(1) 0.0025% dieldrin.

(2) 0.0025% aldrin.

(3) 0.004% gamma-isomer BHC.

(4) 57% sodium arsenite solution diluted to give, in the final wash, 0.1% As_2O_3.

(5) 16% emulsifiable concentrate diluted to give, in the final wash, 0.0025% diazinon.

(6) 3% dieldrin in marble-flour dust of fine consistency, at least 97% of the mixture passing through a 300-mesh B.S. sieve, applied at the rate of 1 lb to five sheep.

SHEEP

In both series of experiments the treated sheep were unshorn September-born Romney lambs carrying, in 1956, a heavy infestation of body lice, and in 1957, a heavy infestation of body lice and a light infestation of keds. Five sheep were in each experimental group in both years except for the arsenic group in 1957, when there were four sheep.

Treatment was on 3 April in 1956 and on 13 February in 1957.

METHOD

In both series of experiments the method was similar. After treatment the sheep were isolated and examined at 48 hours and again at 7 days. They were then isolated for 1 month and re-examined. From this stage, in 1956, the sheep were run together with equal numbers of heavily infested animals, and isolated, in alternate weeks as far as possible, an examination being made at the end of each seven-day interval to ensure that lice had transferred during the contact period and that the effect of at least seven days exposure to the retained dip was observed. In this first series of experiments, one contact period had to be extended to fourteen days owing to a period of continuous rain preventing adequate transfer of lice or adequate examination, in the period beginning 17 weeks after dipping.

In 1957, after the first five weeks, the treated sheep were placed with equal numbers of sheep heavily infested with lice and moderately infested with keds. At the end of 10 and 16 weeks after treatment they were again isolated in their groups for one week and examined. At this point the infestors were needed for other experiments using sheep-dusting machines.

However, by exposing undipped sheep it was possible to have a full group of infestors (one for each treated sheep) at the end of the sixth month after treatment, two of the treatments having been abandoned at the end of seventeen weeks. Twenty-five weeks after treatment, the

TABLE 1. PERIOD OF PROTECTION AGAINST LICE, 1956

Dip	Period After Dipping	% Sheep Infested	No. of Sheep in Group
Arsenic/derris	48 hours	0	5
.00625 BHC	,, ,,	0	5
.00125 BHC	,, ,,	0	5
.00125 aldrin	,, ,,	0	5
.00125 dieldrin	,, ,,	0	5
Arsenic/derris	7 days	0	5
BHC	,, ,,	0	5
BHC	,, ,,	0	5
aldrin	,, ,,	0	5
dieldrin	,, ,,	0	5
After exposure to reinfestation and isolation			
Arsenic/derris	6 weeks	0	5
BHC	,, ,,	0	5
BHC	,, ,,	0	5
aldrin	,, ,,	0	4
dieldrin	,, ,,	0	5
Arsenic/derris	12 weeks	0	4
BHC	,, ,,	0	5
BHC	,, ,,	0	4
aldrin	,, ,,	0	3
dieldrin	,, ,,	0	4
Arsenic/derris	14 weeks	25	4
BHC	,, ,,	40	5
BHC	,, ,,	25	4
aldrin	,, ,,	33	3
dieldrin	,, ,,	20	5
Arsenic-derris	16 weeks	25	4
BHC	,, ,,	100	5
BHC	,, ,,	100	4
aldrin	,, ,,	0	4
dieldrin	,, ,,	0	5
Arsenic/derris	19 weeks	33	3
BHC	,, ,,	100	5
BHC	,, ,,	100	4
aldrin	,, ,,	66	3
dieldrin	,, ,,	80	5
Arsenic/derris	21 weeks	33	3
aldrin	,, ,,	66	3
dieldrin	,, ,,	80	5
Arsenic/derris	23 weeks	33	3
aldrin	,, ,,	66	3
dieldrin	,, ,,	20	5
Arsenic/derris	25 weeks	0	3
aldrin	,, ,,	33	3
dieldrin	,, ,,	0	5

sheep in the remaining groups were again exposed to re-infestation for 4 weeks, isolated for 1 week, and then examined.

In both years the experiments were terminated in September as the lice and ked populations of woolly sheep tend to decline with the onset of spring and the results cannot be accurately assessed.

The sheep used in these experiments were selected poor doers, which are considered to be more prone to infestation. Unfortunately during 1956 four of the twenty-five sheep dipped died, one drowning in a creek and the others dying of general debility. In 1957, one sheep was drowned and one was savaged by dogs and had to be destroyed. In assessing the results, a treatment was considered to have ceased to give complete protection when an adult louse or ked was found on any one sheep in a group after the period of isolation. After the period of complete protection had ended there was a period of partial protection until all of the sheep in the group were infested.

The number of parasites present on sheep, in a group which was classed as being past the end of both complete and partial protection periods, was frequently very small. The results therefore, considering this fact and the high rate of exposure to reinfestation indicate what can be expected of different insecticides under the severest conditions.

An additional test of severity was imposed by the weather in the 1956 trial, rain falling on sixty of the first ninety days, with a total of 17.6 inches in this period.

RESULTS

The full results of the 1956 trial are shown in Table 1 and these are summarised in Table 2.

The results of the 1957 experiments are shown in Table 3 and summarised in Table 4.

TABLE 2. SUMMARY OF RESULTS, 1956

Dip	Period of Complete Protection	Period of Partial Protection
Arsenic/derris	12 weeks	At least 25? weeks
0.00625 BHC	,, ,,	14 weeks
0.00125 BHC	,, ,,	14 weeks
0.00125 aldrin	,, ,,	At least 25? weeks
0.00125 dieldrin	,, ,,	At least 25? weeks

TABLE 3. PERIOD OF PROTECTION AGAINST LICE AND KEDS, 1957

Treatment	Period After Treatment	% Sheep Infested		No. of Sheep in Group
		Lice	Keds	
Arsenic dip 0.1%	48 hours	0	25	4
BHC dip 0.004%		0	0	5
Aldrin dip 0.0025%		0	0	5
Dieldrin dip 0.0025%		0	0	5
Diazinon jet 0.0025%		0	0	5
Dieldrin dust 3%		100	0	5
Arsenic dip	7 days	0	25	4
BHC dip		0	0	5
Aldrin dip		0	0	5
Dieldrin dip		0	0	5
Diazinon jet		0	0	5
Dieldrin dust		100*	0	5
Arsenic dip	5 weeks	0	25	4
BHC dip		0	0	5
Aldrin dip		0	0	5
Dieldrin dip		0	0	5
Diazinon jet		0	0	5
Dieldrin dust		0	0	3‡
After exposure to reinfestation and isolation				
Arsenic dip	11 weeks	0	25	4
BHC dip		0	0	5
Aldrin dip		0	0	5
Dieldrin dip		0	20†	5
Diazinon jet		0	0	5
Dieldrin dust		0	0	3
Arsenic dip	17 weeks	50	75	4
BHC dip		20	0	5
Aldrin dip		0	0	5
Dieldrin dip		0	0	5
Diazinon jet		80	40	5
Dieldrin dust		0	0	3
BHC dip	30 weeks	40	60	5
Aldrin dip		0	0	5
Dieldrin dip		0	0	5
Dieldrin dust		0	0	3

* Nymphs only

† 1 newly emerged unfed ked

‡ 1 drowned, 1 killed by dogs

DISCUSSION

The results in Table 1 may indicate that in the 1956 trial the natural decline in parasites had begun at 23 weeks after dipping and the periods of partial protection shown in Table 2 may therefore be rather exaggerated.

TABLE 4. SUMMARY, 1957

Dip	Period of Complete Protection		Period of Partial Protection	
	Lice	Keds	Lice	Keds
Arsenic 0.1%	11 weeks	0	17 weeks	?
Gamma-BHC 0.004%	11 weeks	17 weeks	30 weeks	30 weeks
Aldrin 0.0025%	30 weeks	30 weeks	—	—
Dieldrin 0.0025%	30 weeks	30 weeks	—	—
Diazinon jet 0.0025%	11 weeks	11 weeks	17 weeks	17 weeks
Dieldrin dust 3.0%	30 weeks	30 weeks	—	—

In the 1957 trial the transfer of keds was always at a low rate being always within the 1–5 range as assessed by the number of live keds on the treated sheep at the end of a contact period. The effect of the long periods of contact between treated and untreated sheep appeared to be that the ked population was never able to increase much on the infestor sheep, owing to the continual migration, and subsequent death, of keds to the treated sheep.

It is possible that the treatments might have broken down in protective value earlier if the reinfestation rate with keds had been higher, but as the ratio of untreated to treated sheep was much higher than is likely to occur under normal conditions, the results are probably a true guide to those likely to be obtained under normal conditions. A higher rate of reinfestation challenge would be desirable where the main object in view was the comparison of insecticides only.

On the basis of these results it appears possible that protection throughout the year against infestation by lice and keds should be obtainable from a single dipping without resorting to unduly high concentrations of insecticide, particularly aldrin and dieldrin.

However, it should be noted that a doubling of insecticide strength does not always result in a doubling of the protection period conferred; for example, reference to the tables of results will show that the difference due to increasing BHC from 0.00125% to 0.004% and to 0.00625% is small.

A comparison of the results of a commercial arsenic/derris/sulphur dip in 1956 with those of the BHC preparations indicate that the former may indeed give a slightly longer period of protection against reinfestation by lice, especially if the BHC dips are allowed to exhaust. The BHC preparations would however be superior for ked control.

CONCLUSIONS

It is concluded that the minimum periods of protection against re-infestation in addition to complete initial kill of lice and keds will be at least three months with any dip registered for sale in New Zealand containing the minimum allowable amounts of BHC (0.004% gamma isomer in the wash). Arsenical dips will give a similar result against lice but are inferior against keds. The observation that arsenical dips are superior to BHC dips against lice probably arises when sheep have been exposed to re-infestation. The end point of protection with BHC apparently occurs sharply at 4 months after dipping whereas the breakdown with arsenic is more gradual.

At least six months protection against re-infestation by lice and keds is to be expected under normal conditions when aldrin or dieldrin dip is used at the minimum strengths permitted (0.0025%) and even when exhaustion does occur the results are likely to be better than with either arsenical or BHC dips at the usual strengths, since there are indications that one twenty-fifth of the strength used here will give a complete initial kill of lice.

Similar results may be possible by dusting with 3% dieldrin, although the rate of application was higher in this experiment than that obtained with commercial sheep-dusting races.

Diazinon, used as an all-over spray to saturation and excess, was inferior in comparison with aldrin and dieldrin used as dips at equal concentrations.

It should be noted that most dips sold in New Zealand contain more than the minimum permissible concentration of insecticide. These results should not therefore be regarded as a direct comparison between one commercial dip containing one insecticide and a second containing another insecticide without reference to the concentration of insecticide in both.

ACKNOWLEDGEMENTS

The assistance of members of the parasitology section at Wallaceville Animal Research Station in making the examinations of the sheep in these experiments and the tolerance and co-operation of the farm staff in managing and feeding these animals is gratefully acknowledged, as is the help of those commercial firms who supplied experimental material. These preparations are not identified in the report since in most cases they were not used at the concentrations recommended by the manufacturers.

THE CONCENTRATION OF RADIOACTIVE IODINE BY THE THYROID GLANDS OF NORMAL SHEEP UNDER VARIOUS CONDITIONS

By EVAN WRIGHT, Animal Research Station, Department
of Agriculture, Wallaceville.

(Received for publication, 29 November 1957)

Summary

Training sheep in the handling procedure for measurement of radioactivity and varying the site of injection of radioiodine did not affect the radioiodine concentrating power of their thyroids.

On the other hand 100 μg carrier iodine added to the radioiodine increased the maximum level in the gland by about 30%.

Pregnant ewes accumulated 25% less radioiodine than empty ewes.

The amount of radioiodine absorbed by the thyroid gland was inversely related to the mean ambient temperature.

INTRODUCTION

Sinclair and Andrews (1954) have reported congenital goitre in lambs from ewes that had been fed on thousand-headed kale at Manutuke Animal Research Station. Though the dams of the goitrous lambs appear to be normal, it is unlikely that they remain entirely unaffected by conditions that produce severe goitre, and often death, in the lambs. If this is true, examination of the thyroid function of the ewes should provide a better test of the goitrogenic property of the feed than the appearance of goitrous lambs once a year. Measurement of radioiodine concentration can be used to investigate thyroid function without loss of the animals and this method was chosen for the investigation of the goitrogenic effect of thousand-headed kale.

Though much has been written about the absorption of radioactive iodine in human beings and in laboratory animals, little is known of factors that may affect its accumulation in the thyroid gland of the sheep. Therefore, before the method was applied to the investigation of the goitrogenic property of kale, a series of experiments was made on a group of normal sheep under a variety of conditions.

EXPERIMENTAL METHODS

The measurements of radioactivity were made with a gamma-ray counter having a lead-filled copper cathode (G10Pb 20th Century Electronics). This counter is enclosed in a lead castle 3.75 cm thick, having a window 2.0 cm wide by 7.5 cm long over the sensitive part of the counter. The castle is mounted in the crush shown in Fig. 1a so that this window is uppermost, and light is excluded by a 0.6 mm aluminium-alloy cover.

The counter and castle are pivoted about their longitudinal axis so that they may follow the movements of the sheep, whose thyroid is held

N.Z. J. agric. Res, 1: 224–230.

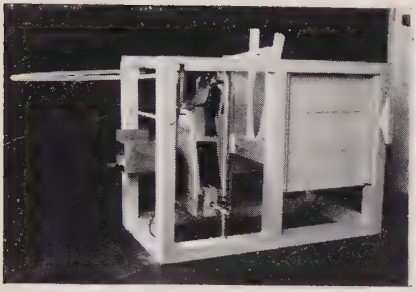


Fig. 1a.

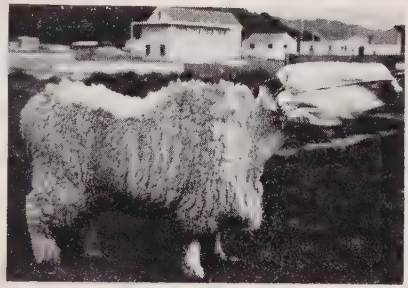


Fig. 1b.

Fig. 1.—Apparatus for the measurement of thyroidal radioiodine in the sheep.
(a) The crush and counter
(b) The head bag

31.5 cm above the counter tube by a neck holder. To place the sheep in the crush, one first straps a shaped and ventilated bag over its head as seen in Fig. 1b. The tapered front end of this bag has a steel frame to which is attached the lead rope, by means of which the sheep is led into the crush and tethered to the projecting iron rod. The animal's neck is placed in the shaped neck rest and secured by a strap and sponge-rubber pad, the whole unit of castle and rest being held forward by a rubber spring, so that the curved front of the holder is against the sheep's jaws. Each time an animal is placed in the crush the position of its thyroid is checked by palpation of the larynx from below.

The apparatus also has a neck yoke and a rope at the rear for the control of the few unruly animals, but is usually used without these. The headbag is very effective in controlling nervous and unruly sheep. When they first have the bag on their heads they are disinclined to move, but soon gain confidence and may be led about quite easily. A little training in the procedure is a distinct advantage, but the method has been used satisfactorily on young animals which were not long off the hills, and which were not accustomed to handling or even to the presence of men and dogs. While ewes are quieter at first than wethers, the latter are more quickly trained; in both cases gentle handling is rewarded.

The dose of radioiodine is measured in the phantom thyroid shown in Fig. 2. This consists of a polyethylene vessel made to fit the neck holder and filled with water. The test tube (internal diameter 1.1 cm) containing the radioiodine in 1.5 ml of solution is placed in the vessel so that there is 1 cm of water below it. This standard gives a count of about 40 per second for a dose of 70 μc.

Because the phantom is not an exact replication of a sheep thyroid, the calculated fraction of the dose absorbed by the gland is only approximate, but since all experiments are referred to measurements on the same standard, corresponding figures may be directly compared.

Except where it is otherwise stated, the dose of 70μc of carrier-free radioiodine was given intraperitoneally, low down on the right side.

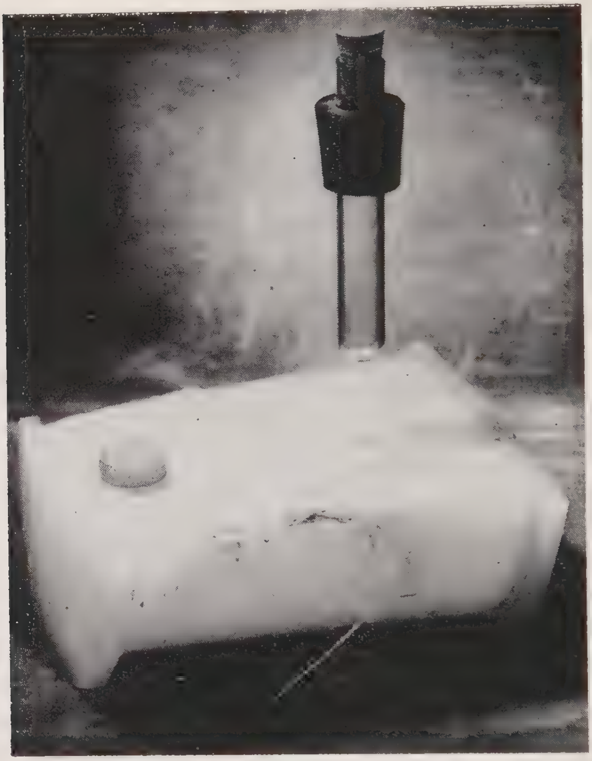


Fig. 2.—Phantom sheep thyroid.

The thyroidal radioactivity was usually measured at short intervals for 48 hours and then twice daily for at least two weeks. The counting period of five minutes gave a standard error of less than 1.5% for the higher counts, but considerably more on the lower ones.

For these studies a group of twelve ewes was used. These sheep, which were in their third year at the beginning of the experiments, were grazed on normal pasture available on the station with the addition of hay at some periods. During the radioiodine absorption measurements the sheep were held indoors without food (but with access to water) for the first 48 hours and after that were brought in daily for measurement.

RESULTS AND DISCUSSIONS

The Effect of Training

Both endogenous and exogenous adrenaline inhibit the thyroid absorption of radioiodine (Badrick *et al.* 1954), and in the rabbit this effect may be produced by "restraint" (Brown-Grant and Gibson 1956). Since repeated measurements were to be made on the same animals it was advisable to find out whether training in the handling procedure had any effect on the accumulation of radioiodine.

Hence in the first experiment the twelve sheep were brought in daily and drafted into two groups. One group was left quietly in the yard while the others were trained by being put into the crush and held there for five minutes. After this both groups were returned to the pasture together. This procedure was followed for three weeks before the measurement of the accumulation of radioiodine.

The results of these measurements are plotted in Fig. 3. Time is shown on the abscissa, and thyroidal radioactivity, expressed as a percentage of the dose, on the ordinate. The number of sheep in the group is shown in the circle and this practice is followed in the graphs for following experiments.

The slight difference between the two groups is nowhere significant at the 10% probability level.

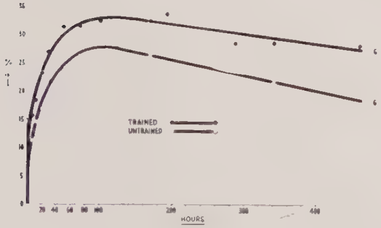


Fig. 3

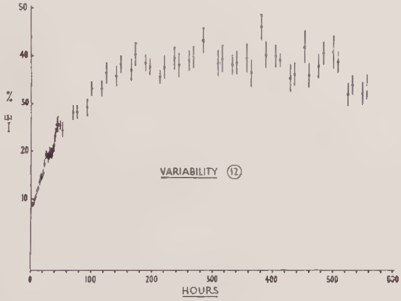


Fig. 4

Fig. 3.—The effect of training.
Fig. 4.—The variability in radioiodine concentration.

The Variability Between Sheep

In the next experiment all twelve animals were treated alike and measurements made at more frequent intervals. In Fig. 4 the results are plotted as perpendicular lines one standard error to each side of the mean.

The Effect of Varying the Site of Injection

In routine radioiodine-concentration measurements the intraperitoneal route of injection is to be preferred because of its ease of application and the low risk of radioactive contamination. However, the possibility should not be neglected that, when using this route, one might be measuring some rate of dispersion or absorption other than that by the thyroid.

The results of the third experiment, given in Fig. 5, show that the intraperitoneal route is not inferior to the intravenous. On the contrary it gave a slightly, though not significantly, greater concentration than did either the intravenous or subcutaneous routes. This is in agreement with other reports concerning work on rats (Badrick *et al.* 1954; Chakraborty 1956).

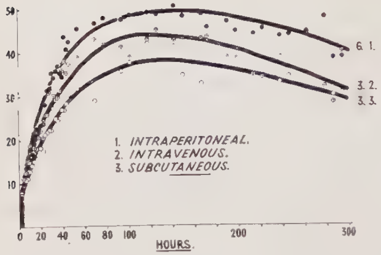


Fig. 5

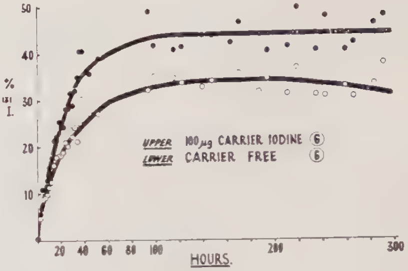


Fig. 6

Fig. 5.—The effect of varying the site of injection.

Fig. 6.—The effect of adding carrier iodine.

The Effect of Carrier Iodine

Other experimenters have shown that, in rats, the addition of inactive iodide to the radioiodide reduces the proportion absorbed (Fish *et al.* 1952; Mann *et al.* 1949) but in my experiment the opposite effect was produced. In Fig. 6 the curve for the group receiving 100 μg carrier iodide has a maximum value 30% greater than the carrier-free group. An analysis of variance shows that the difference is statistically significant at the 10% probability level at all times and at the 5% level from 20 to 120 hours.

Two factors may have a bearing on this effect. Firstly, the level of inactive iodide used is physiologically small compared with that used by others. Nachimson and his colleagues (1954) produced a slight effect by 10 μg of iodide in rats and similar results were obtained by Wolff and Chaikoff (1948). Corresponding to this low level for rats the dose for sheep on the basis of body weight would be about 2 mg and on the basis of the daily requirements for iodine (rat 2 μg, sheep 100 μg, Underwood 1956) it would be 500 μg. It is therefore unlikely that 100 μg of iodine would exercise any inhibition. The second possible factor is thiocyanate. In my experience and in that of Dr. Butler at Grasslands Division, Palmerston North, most sheep when grazing pasture have a level of serum thiocyanate of 1–2 mg/100 ml, probably derived from the cyanogenic glucosides of white clover (Flux *et al.* 1956). When an animal receives thiocyanate for a long time, the radioiodine curve may be normal, but if the thiocyanate is withdrawn just before the radioiodine test the maximum accumulation in the thyroid is increased (Butler *et al.* 1957). As it is well known that iodine will overcome the effect of thiocyanate the carrier iodine given in my experiment may have had a result similar to that produced by the withdrawal of thiocyanate.

The Effect of Pregnancy

There are conflicting reports on the interference of female sex hormones on radioiodine accumulation. For instance, Soliman and Reineke (1954) found a cycle of absorption following the oestrous cycle, and Feldman (1956) increased the iodine accumulation in the glands of castrate male rats by the administration of oestrogen. On the other hand Paschkis *et al.* (1948) observed no effect in rats, after ovariectomy or injection of oestrogen and are supported by the work in rabbits of Brown-Grant (1956) who, however, reported an increase both in absorption and release in very late pregnancy. At an earlier stage, his graphs show a decrease of a few per cent but this was not significant. In my experiment I found that the pregnant ewes varied markedly from the controls. The six ewes that were put to the ram were 58–76 days pregnant when the measurements shown in Fig. 7 were made. The lowering of the thyroidal concentration of radioiodine by pregnancy amounted to about 20% at the maximum point and was significant at the 5% probability level from four hours onward.

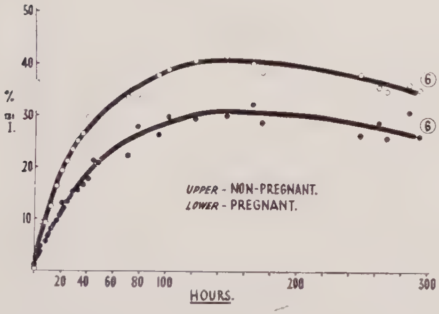


Fig. 7

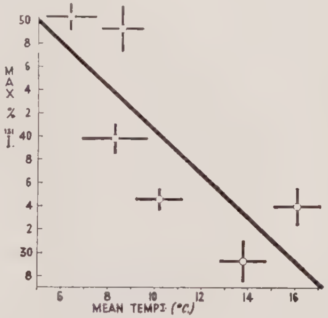


Fig. 8

Fig. 7.—The effect of pregnancy.
Fig. 8.—The influence of ambient temperature.

The Effect of Temperature

Blincoe and Brody (1955) have made a brief review of the literature on the relationship of thyroidal activity and ambient temperature in several species and have added their observations on cattle. Results obtained by various methods are in agreement with the hypothesis that thyroidal activity is inversely related to ambient temperature.

Since these experiments were made on the same animals at various seasons, they provide an opportunity to compare these two variables in the case of sheep. The relationship is shown in Fig. 8. On the ordinate is plotted the mean, and standard error of all values of thyroidal radioactivity observed for control animals between the 96th and 144th hour after injection of radioiodine; on the abscissa the average, and standard error, of mean daily temperatures recorded at Wallaceville from the time of injection to 144 hours later—that is to say, over the whole period of accumulation. The six points represent the five experiments reported above and one other which duplicated the second experiment. The correlation of maximum absorption and ambient temperature is significant at the 5% level of probability and the regression co-efficient of —1.86% per degree centigrade, which was used to drawn the line on the graph, is close to that calculated from figures given by Blincoe and Brody (1955) for cattle, namely —1.1 to —1.7.

Acknowledgements

I am indebted to Mr. I. W. Bayne and Mr. B. J. Roddick for technical assistance and to Mr. A. L. Bryant for the illustrations.

References

Badrick, T. E.; Bramblecombe, R. W.; Reiss, J. M.; Reiss, M. 1954: The Influence of Stress Conditions on the Uptake of ^{131}I by the Rat Thyroid. *J. Endrocrin. 11:* 305–13.

Blincoe, C.; Brody, S. 1955: Environmental Physiology and Shelter Engineering xxxii. The Influence of Ambient Temperature, Air Velocity, Radiation Intensity and Starvation on Thyroid Activity and Iodine Metabolism in Cattle. *Univ. Missouri Agric. exp. Sta. Res. Bull. 576.* 36 pp.

Brown-Grant, K. 1956: Gonadal Function and Thyroidal Activity. *J. Physiol. 131:* 70–84.

Brown-Grant, K.; Gibson, J. G. 1956: The Effect of Exogenous and Endogenous Adrenalin on the Uptake of Radioiodine by the Thyroid Gland of the Rabbit. *J. Physiol. 131:* 85–101.

Chakraborty, K. P. 1956: Variations in Uptake and Excretion of Radioiodine in the Rat Following Different Modes of Administration. *J. Endocrin. 13:* 354–9.

Feldman, J. D. 1956; Effect of Oestrogen on Thyroid Uptake of ^{131}I in Adrenalectomised Rats. *Amer. J. Physiol. 184:* 369–70.

Fish, W. A.; Carlin, H.; Hickey, F. C. 1952: The Thyroidal Uptake, Retention and Iodine Pool in Rats under Controlled Iodide Diet. *Endocrinology 51:* 282–92.

Flux, D. S.; Butler, G. W.; Johnson, J. M.; Glenday, A. C.; Petersen, G. B. 1956: Goitrogenic Effect of White Clover (*Trifolium repens* L.) *N.Z. J. Sci Tech. A38:* 88–102.

Butler, G. W.; Flux, D. S.; Petersen, G. B.; Wright, E. W.; Glenday, A. C.; Johnson, L. M. 1957: Goitrogenic Effect of White Clover (*Trifolium repens* L.) II. *N.Z. J. Sci. Tech. A38:* 793–802.

Levine, H.; Remington, R. E.; Vonkolnitz, H. 1933: Studies on the Relation of Diet to Goitre. II. The Iodine Requirement of the Rat. *J. Nutr. 6:* 347–54.

Mann, W.; Bale, W.; Hodge, H. C.; Warren, S. L. 1949: The Distribution in Rabbit Tissue of Intravenously Injected Iodine as Shown by the Radioisotope I^{130}. *J. Pharmacol. exp. Therapy 95:* 12–17.

Nachimson, H. J.; Benson, R. H.; Szafir, J. J.; Turner, R. B.; Allen, H. C.; Talmage, R. V. 1954: Comparative Studies of Effects of Phenothiazine and Iodide on Thyroid Uptake of I^{131}. *Proc. Soc. exp. Biol. Med. 87:* 157–62.

Paschkis, H. E.; Cantarow, A.; Peacock, W. C. 1948: The Influence of Oestrogens on Thyroid Functions as Measured by Uptake of Radioactive Iodine. *Proc. Soc. exp. Biol. Med. 68:* 485–6.

Sinclair, D. P.; Andrews, E. D. 1954: Goitre in New Born Lambs. *N.Z. vet. J. 2:* 72–9.

Soliman, F. A.; Reineke, E. P. 1954: Changes in Uptake of Radioactive Iodine by the Thyroid of the Rat during the Oestrus Cycle. *Amer. J. Physiol. 178:* 89–90.

Underwood, E. J. 1956: "Trace Elements in Human and Animal Nutrition." p. 259. Academic Press N.Y. 430 pp.

Wolff, J.; Chaikoff, I. L. 1948: The Inhibiting Action of Iodide on Organic Binding of Iodine by the Normal Thyroid Gland. *J. biol. Chem. 172:* 855–6.

SUPERFICIAL SCALD
SOME METHODS OF CONTROL, REPORTED OVERSEAS, APPLIED TO NEW ZEALAND GROWN GRANNY SMITH APPLES

By C. S. Padfield, Fruit Research Division, Department of Scientific and Industrial Research, Havelock North

(Received for publication, 23 September 1957)

Summary

Satisfactory control of blotchy superficial scald on Granny Smith apples held in cool store for 200 days can be obtained with oiled wraps containing 10–12% oil. After 200 days storage, partial control of senescent scald was obtained using wraps containing 15 to 20% oil. Re-wrapping fruit with fresh oiled wraps after 3 months storage did not aid scald control. Reduction of temperature from 37–38°F to 31–32°F after 4 weeks' storage did not assist scald control. Destruction of cuticle by physical means increased scald and had a deleterious effect on the appearance of the fruit. Wiping apples with ethyl alcohol reduced scald in one season, but had no effect the following year. A spray programme using captan as a fungicide in place of sulphur had no effect on subsequent development of scald on stored apples.

Introduction

The physiological storage disorder of apples, superficial scald, is universally known. The most widespread method adopted for its control is the wrapping of apples in oiled paper, first reported by Brooks *et al.* (1919). Sometimes oiled wraps failed to give economic control of scald. For this reason investigators have been searching for improved means of control, either satisfactory in themselves, or as aids to the method of using oiled wrapping paper.

A successful method reported from Western Australia for controlling scald on Granny Smith apples by temperature reduction from 40°F, after 4 weeks, to 32°F was reported by Melville and Hardisty (1953). Shutak *et al.* (1953) were successful in reducing scald using methods that wholly or partially destroyed the cuticle. Haller (1954) quoted a claim that a reduction of scald might be expected if apples received a fungicidal spray programme incorporating captan (n-trichloromethyl-thio tetrahydropthalmide). Marshall (pers. comm. 1955) gave results that were conflicting as to the value of this method. Padfield (1950) has shown that susceptibility of the Granny Smith apple to scald was induced relatively early during storage life, although scald might not be manifest until much later. It was thought that if the oiled wraps were removed and exchanged for fresh ones, after 3 months storage, added control of scald might be obtained. Brooks *et al.* (1919) had specified that for satisfactory control of scald, 15% by weight of oil was necessary in each paper. For many years New Zealand has used Canadian wraps containing approximately 15% oil. In 1954 a cheaper line of Swedish wraps became available, but as the oil content was only 10–12% by weight it was necessary to find out if these would give effective control of scald. Trials to determine this point were undertaken during 1954 to 1956.

EXPERIMENTAL

Reduction of Temperature, Change of Wraps, Oil Content

1954

Samples of Granny Smith apples were obtained from two orchards, No. 1 where the variety was known to be harvested early, 13 April, No. 2 considerably later, 1 May. Two examinations were made. At each, 4 bushels of count 113 (orchard No. 1) or count 100 (orchard No. 2) were examined from every treatment. Treatments were as follows:

1. Plain sulphite wraps
2. Swedish oiled wraps (11.9% oil)
3. Canadian oiled wraps (15.9% oil)
4. Canadian oiled wraps. Initial wraps removed after 3 months and replaced with fresh wraps.

TABLE 1. PER CENT SUPERFICIAL SCALD ON GRANNY SMITH APPLES, 1954
(Means of 4 bushel samples)

	Orchard No. 1				Orchard No. 2			
	1st Examination		2nd Examination		1st Examination		2nd Examinatio	
	After 174 days storage		After 230 days storage		After 163 days storage		After 216 days storage	
Treatment	Slight	Severe	Slight	Severe	Slight	Severe	Slight	Severe
1* Plain sulphite wraps	42	11	29	70	24	7	36	46
2* Swedish oiled 11.9% wraps	1	0	59	21	0	0	37	2
3* Canadian oiled 15.9% wraps	0	0	56	9	0	0	27	1
4* Canadian oiled wraps, replaced after 3 months	0	0	53	25	0	0	25	2
5 Canadian oiled wraps. 4 weeks 37–38°F, remainder 31–32°F	0	0	60	19	0	0	32	2

* Fruit was stored at 31–32°F continuously.

5. Canadian oiled wraps. Fruit held for first 4 weeks at 37–38°F, subsequently moved to 31–32°F for remainder of the storage period. Results are presented in Table 1.

At the various examinations in this and subsequent experiments, fruit was classified into three categories: clean; slight scald, being fruit that showed scald but was considered marketable; severe, all fruit marked to the extent of making it too unattractive to market.

It is of interest to note that the type of scald recorded at the first examination was all of the heavily blotchy, rugose type (Fig. 1). At the second examination, scald showing on the fruit of treatment 1 was of two kinds, the same rugose type and the senescent shadow type (Fig. 2), whereas scald on all oiled-wraped treatments was of the shadow type. In addition, all samples at the second examination were suffering from severe breakdown and coreflush: in fact, they had been held to the point of destruction. After removal from cool store, before each examination, all fruit was held for 14 days at 60–65°F or at air temperature, whichever was the higher. On removal from store, prior to the second examination, no senescent scald was showing. At this time also the rugose scalded areas on check samples were badly infected with secondary fungus. No fungus was showing on any of the other treatments.

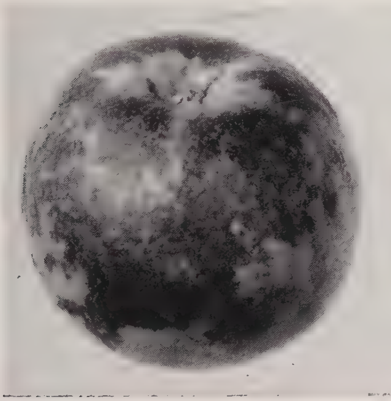


Fig. 1

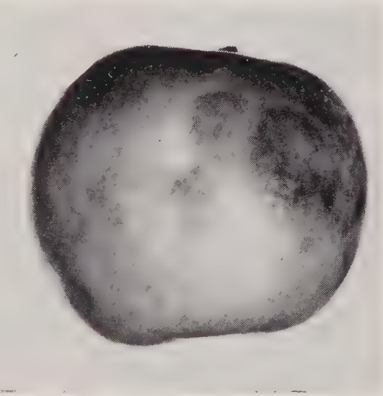


Fig. 2.

Fig. 1.—Severe, rugose, blotchy superficial scald.
Photo: H. E. Powell

Fig. 2.—Severe, senescent, shadow-type superficial scald.
Photo: H. E. Powell

1955

The experiment was repeated with the exception of treatment 4, because this method did not show promise and would involve a large increase in handling charges. Canadian wraps used contained the exceptionally high content of 23.4% oil. The Swedish wraps were the residue left over from the previous year, and their oil content had dropped to 10.7%.

Fruit from orchard No. 1 was harvested on 26 April (later than in 1954), and that from orchard No. 2 on 27 April. As picking dates were unavoidably close together, this experiment become a comparison between two sets of samples under different management, instead of a comparison between maturities and orchards. Samples were of similar size, those from orchard No. 1 being all 100 count per bushel, from orchard No. 2 they consisted of two bushels of count 100 and 1 bushel each of counts 113 and 125. Results are presented in Table 2.

TABLE 2. PER CENT SUPERFICIAL SCALD ON GRANNY SMITH APPLES, 1955
(Means of 4 bushel samples)

	Orchard No. 1				Orchard No. 2			
	1st Examination		2nd Examination		1st Examination		2nd Examination	
	After 155 days storage		After 209 days storage		After 154 days storage		After 209 days storage	
Treatment	Slight	Severe	Slight	Severe	Slight	Severe	Slight	Severe
1* Plain sulphite wraps	37	28	29	68	32	31	26	69
2* Swedish oiled wraps, 10.7%	1	0	70	8	7	1	58	6
3* Canadian oiled wraps, 23.4%	0	0	70	13	3	0	34	3
5 Canadian oiled wraps, 4 weeks 37–38°F, remainder 31–32°F	1	0	69	16	0	0	12	0

* Fruit was stored at 31–32°F continuously.

The appearance of the fruit at the two examinations was similar to that described for 1954. The interesting result was the one obtained at the second examination under treatment 5 from orchard No. 2. Each of the four bushels in this sample showed approximately the same amount of scald.

1956

In view of the one promising result obtained in 1955, it was decided to repeat treatments 2 and 5. Oil content of wraps used this year was 13.9%. There were no further supplies of Swedish paper available, the mill having closed during the period of the trial. In previous years any form of oiled wrap had proved superior to plain paper, and this treatment was abandoned. The supply of fruit was changed to a new orchard, No. 3, on more fertile soil, the trees being superior in vigour and cropping capacity. Harvesting took place on 29 April and samples of even size were selected, each bushel containing 113 apples. Results are presented in Table 3.

TABLE 3. Per Cent Superficial Scald on Granny Smith Apples, 1956
(Means of 4 bushel samples)

Treatment	Orchard No. 3			
	1st Examination		2nd Examination	
	After 155 days storage		After 205 days storage	
	Slight	Severe	Slight	Severe
3 Canadian oiled wraps. 13.9% oil. 31–32°F continuously	5	1	39	30
5 Canadian oiled wraps 13.9% oil. 37–38°F for 4 weeks, remainder 31–32°F	4	0	39	30

The small amount of scald recorded at the first examination was again the rugose type, while, at the second, 5% in treatment 3 and 1% in treatment 5 were also of this type, the remainder being the senescent shadow type.

The results for the three seasons indicate that for storage periods up to seven months, oiled wraps containing 10 to 23% of oil were quite satisfactory for the control of rugose blotchy scald. None of the oiled wraps used gave commercial control of senescent shadow scald during the last two months of a seven-month storage period. There is, however, an indication that a positive correlation exists between oil content of wraps and control of senescent scald. Although re-wrapping the fruit after three months with fresh paper was tried for one season only, the result obtained suggests that there is no merit in this method, particularly when taking into consideration the extra work entailed.

The Western Australian method of controlling scald by storing at relatively high temperature for the first month proved of no value over the standard method of storage (treatment 3), except in the one instance already cited. Again, there would appear to be no merit in this procedure for Hastings-grown Granny Smith.

Destruction of Cuticle

Shutak *et al.* (1953) showed that if cuticle could be destroyed by physical abrasion or by alcohol as a solvent, incidence of scald could be considerably reduced. As a means of abrasion, they passed apples over the dry brushes of an apple washer and found that a maximum control was obtained if this procedure was repeated six times.

Apple washing machines are not used in New Zealand, but it was thought that the rollers of a screw-type fruit-sizing machine would make a suitable substitute.

1955

Apples picked on 3 May were obtained from orchard No. 3 and treated as follows:—

1. Check, no special treatment.
2. Passed over a Benseman grader 6 times.
3. Rubbed with cloth saturated with 95% alcohol.

Treatment samples consisted of 4 bushel cases of count 113. The roller-type grader relies for its sizing on a widening gap between two rotating coarse threaded rollers, with the smaller apples passing into the bins first. The movement of the rollers also causes the apple to rotate, so that by the time the apples reach the bins they are well polished. It will be appreciated that apples of size 113 count would receive a thorough brushing from a six-fold treatment. After treatment, all apples were packed in plain paper to allow a maximum of scald to develop. The fruit was stored at 31–32°F and examined on 27 October, after 177 days from harvest (this included 14 days post-cool-storage at 60–65°F). Results of this examination are presented in Table 4.

TABLE 4. PER CENT SUPERFICIAL SCALD ON GRANNY SMITH APPLES WITH CUTICLE DESTROYED
(Means of 4 bushel samples)

	Orchard No. 3			
	1955		1956	
	After 177 days storage		After 160 days storage	
Treatment	Slight	Severe	Slight	Severe
1 Check	27	18	22	64
2 Passed 6 times over rollers of a Benseman grader	38	34	—	—
3 Wiped with cloth saturated with 95% alcohol	36	10	25	59

On removal from store in mid October none of the fruit had developed scald, but it developed effectively during the post-cool-storage treatment.

Scald on the check fruit mostly occurred on the cheeks of the apples, and was blotchy and dark brown in colour. The appearance of apples run over a grader six times was very much inferior to that of fruit under the other two treatments. Scald was most intense on any areas where the fruit had been marked, bruised, or scratched during treatment. Although considerable care was taken when handling the apples, it was obvious that the method was too harsh to be of practical value. and that any method of abrasion would have to be far gentler in its physical effect on the fruit. If the treatment did nothing else, it provided a classic example of the detrimental effect that bruising and excessive handling can have on the out-turn of cool-stored apples.

The apples wiped with alcohol were by far the best in appearance. Although the total number of scalded apples was approximately the same in treatments 1 and 3, the results shown in Table 4 are misleading and need qualification. The intensity of the scald in treatment 3 was so slight that in most of the slight category the discolouration was merely a light browning of very small areas. It took two forms. One of these consisted of small circular areas about the size of a threepenny piece, that occurred at points of contact between apples; there was generally only one of these per apple. The other showed as a slight discolouration within the calyx or stalk cavity, which suggested that these areas scalded because alcohol had not reached them. There is no doubt that this treatment was successful in reducing the intensity of scalding, even if the number of apples showing scald was not reduced.

1956

In order to verify the result obtained in 1955 with treatment 3, a further trial was carried out. Treatment and sample size were the same in all respects as for the previous year. Fruit was picked on 2 May and examined 160 days later on 9 October. Results are also shown in Table 4.

Scald on both samples was severe, being identical in pattern. Lesions were irregular and blotchy and brown; they extended over large areas of the skin and were not confined to calyx or stalk cavities. In this instance wiping with alcohol was of no value. It would appear that this method of treatment cannot be relied on, and for this reason would have no practical application under commercial conditions.

Reduction of Scald by Captan

In 1956, a small block of trees on orchard No. 2 was subdivided so that two spray programmes could be applied, guard trees surrounding the trees to be sampled were arranged to obviate spray drift. One set of trees received a programme of alternating thiram (95% at $\frac{1}{2}$ lb/100 gal) and colloidal sulphur (40% at 2 lb/100 gal), nine sprays in all; the other set received ten applications of captan (3 lb/100 gal to closed calyx, thereafter at 2 lb/100 gal) and no other fungicide. On 24 April a composite sample of 16 bushels was picked from six trees under each spray programme. Eight bushels of each were wrapped in plain sulphite wraps, the remaining eight in oiled wraps containing

TABLE 5. EFFECTS OF TWO SPRAY PROGRAMMES ON INCIDENCE (%) OF SUPERFICIAL SCALD ON GRANNY SMITH APPLES
(Means of 4 bushel samples)

	Orchard No. 2							
	1st Examination				2nd Examination			
–	162 Days from Harvest				204 Days from Harvest			
Spray Programme	Plain wraps		Oiled wraps		Plain wraps		Oiled wraps	
	Slight	Severe	Slight	Severe	Slight	Severe	Slight	Severe
Sulphur Thiram	38	14	13	1	23	63	18	13
Captan	32	13	16	2	27	66	24	23

13.9% of oil. The fruit was large, being all of count 88 to the bushel. Samples were held at 31–32°F. followed by 14 days at 60–65°F. before examination. Half of each sample was examined on 2 to 4 October, after 162 days from harvest, the remainder on 13 to 14 November, after 204 days. Results of these examinations are shown in Table 5.

Scald observed at the first examination was all of the blotchy type, while at the second examination both the blotchy and the shadowy senescent type were present. No attempt was made to record the two types separately. At the second examination the fruit was in the last stages of senescence, showing severe coreflush and considerable breakdown.

These results show that captan applied at the rates recommended in a normal spray programme does not control scald under Hastings conditions

REFERENCES

BROOKS, C.; COOLEY, J. S.; FISHER, D. F. 1919: *J. agric. Res. 18:* 211–40.

——————1923: *U.S. Dept. Agric. Fmrs Bull. 1380.*

HALLER, M. H. 1954: *Ibid. 1380.*

MELVILLE, F.; HARDISTY, S. E. 1953: *J. Agric. W. Aust. (3rd Ser.) 2:* 101-18.

PADFIELD, C. A. S. 1950: *N.Z J. Sci. Tech. A32:* 45–7.

SHUTAK, V. G.; CHRISTOPHER, E. P.; PRATT, L. C. 1953: *Proc. Amer. Soc. hort. Sci. 61:* 228–32.

A REVIEW OF WORK IN NEW ZEALAND ON THE CLOVER CASE-BEARERS *Coleophora spissicornis* Haw. AND *C. alcyonipennella?* Kollar (Coleophoridae, Lep.)

By A. M. STUART, Entomology Division, Department of Scientific and Industrial Research, Nelson

(Received for publication, 16 October 1957)

Summary

The geographical distribution of *Coleophora spissicornis* Haw. and *Coleophora alcyonipennella?* Kollar as pests of clover is given.

Attempts to control the pests in New Zealand are discussed and the difficulties explained.

Observations on the life cycles of *C. spissicornis* and *C. alcyonipennella?* are made and the latter species is found to have four instars.

The genitalia of the adults of *C. spissicornis* and *C. alcyonipennella?* are figured and a means of distinguishing between the final instar larvae of the moths is given.

INTRODUCTION

Species of *Coleophora,* case-bearer moths, are now known to attack clovers in New Zealand, Australia, Tasmania, and the United States as well as in Europe and Asia Minor. In the United States and New Zealand the moths are considered to be pests as they are responsible for much damage to commercial seed crops of white clover *Trifolium repens* Linn. While they are quite common in the other countries they are not of economic importance since clovers are not extensively grown there for seed.

The two species of *Coleophora* known to be of economic importance in clover seed crops are *C. spissicornis* Haworth, which is known to occur in U.S.A., Australia, Tasmania, and New Zealand, and which is indigenous to Europe and Asia Minor, and *C. alcyonipennella*? Kollar, which is present in Australia, Tasmania, and New Zealand. The latter species is probably also indigenous to Europe (Bradley, pers. comm.).

This paper will be confined to the two species of *Coleophora* occurring in New Zealand, and an account of our present knowledge of these pests will be given together with short descriptions of the final instar larvae and genitalia of the adults.

OCCURRENCE

New Zealand

The first record of a coleophorid in New Zealand was in 1922 at Governor's Bay near Christchurch (Lindsay 1927) when a single specimen of *C. spissicornis* (*C. fabriciella* Villiers) was taken. Nothing more was heard of coleophorids until 1940 when damage to white-clover crops at Hastings was reported (Hamilton, 1944). Dumbleton (1952) discovered that another species of *Coleophora* was present in New Zealand attacking the seed of white clover and he found that this second species, which he identified as *C. frischella* Linn. was as important as, if not more important than, *C. spissicornis.* The host list as known of the two species in New Zealand is:

C. spissicornis.	White clover	*Trifolium repens* Linn.
C. alcyonipennella?	White clover	*T. repens* Linn.
	Strawberry clover	*T. fragiferum* Linn.

United States

C. spissicornis was first reported to be a pest in California of white clover (Ladino) in 1952 and it has now spread to the eastern states (Smith and Andres, 1954). These authors have also recorded *C. spissicornis* from *Trifolium pratense* Linn. and *T. arvense* Linn. in U.S.A. No occurrences of *C. alcyonipennella?* are recorded in America.

Denmark

Hammer (1937) has given the only reference to date of a coleophorid doing economic damage to a clover crop in Europe. This was an isolated attack by *C. spissicornis* on a red-clover (*T. pratense*) crop in eastern Zealand. *C. spissicornis* had however been known to occur in large numbers in Europe as long ago as 1908 according to Hammer.

Australia

There have been no reports of economic damage by coleophorids to clover from Australia, though *C. alcyonipennella?* occurs there. There has been some confusion in the nomenclature of *C. alcyonipennella*? in New Zealand and Australia. It was known for a long time (Lindsay 1927) that *C. spissicornis* occurred in New Zealand. Dumbleton (1952), however, found the other species which he described as *C. frischella.* He discovered that this second species also occurred in Tasmania, where it had been identified as *C. deauratella* Zell. (Turner in Meyrick, 1928). This has been confirmed by the present author and it is also known that the same species is present in Australia as moths sent to Dr. I. Common, C.S.I.R.O. Melbourne, were compared with specimens in Australia. However, the moth is neither *C. frischella* L. nor *C. deauratella* Zell, but it is now considered by J. D. Bradley of the British Museum (N.H.) to be the true *C. alcyonipennella*? Kollar (pers. comm.). Until this is definitely proved, however, the moth is being known as *C. alcyonipennella*?

It should be noted that in Meyrick (1928) *C. alcyonipennella*? runs to *C. deauratella* in the key, being separated from *C. frischella* by size; the figures in Pierce and Metcalfe (1935) are not sufficiently clear to correct this mistake. It should also be noted that the figure of *alcyonipennella?* in Pierce and Metcalfe is really that of *paripennella* Zell. (Bradley, 1956).

BIOLOGICAL CONTROL IN NEW ZEALAND

In 1950, at the request of the New Zealand Government, the European Laboratory of the Commonwealth Institute of Biological Control began work in Belgium and France to discover what parasites attacked the moths which at this time had been identified as *C. spissicornis* Haw. and *C. frischella* L. It proved difficult to find *spissicornis* and their efforts were then confined to the moth identified as *frischella* L. which was much more abundant. Nine thousand parasitised larvae of *C. frischella* were received from Europe in 1952; most of these were distributed outdoors still unemerged in March 1953. Liberations of adults of two species of parasites were made near Hastings (in Hawke's Bay) and Nelson in February 1953. These were 200 *Habrocytus milleri* Verbeke and Delucchi in Hawke's Bay, and several hundred *H. milleri* and 100 *Bracon osculator* Nees in Nelson; both these species are hyper-parasites. Whether emergences occurred from this material is not known. Active work on this project in New Zealand ceased and was not resumed until 1955. Then, on comparing work done by the C.I.B.C. with what was known here, it appeared that *frischella* in New Zealand had either changed its habits (e.g. no evidence was seen of its feeding on *Melilotus altissima* Thuill even though this is its main host in Europe and, indeed, was the plant from which the C.I.B.C. were collecting their material) or else the insect had been misidentified. On consulting Pierce and Metcalfe (1935) and Meyrick (1928) it seemed that a misidentification had occurred. N.Z. specimens of the moth known as *frischella* were then sent to the British Museum (N.H.) who at first considered them to be *frischella* but on a further examination of more specimens they were found to be *C. alcyonipennella*? Kollar.

TABLE 1. EMERGENCES OF PARASITES OF *C. frischella* Linn. IN NEW ZEALAND DURING SEPTEMBER-DECEMBER 1955 FROM LARVAL MATERIAL COLLECTED AT MONTMEDY, FRANCE

Determinations made by the British Museum (N.H.)

Species	Numbers	
	Males	Females
Ephialtes brevicornis (Grav.)	7	6
Habrocytus milleri Verb. et Del.	2	7
Angitia sp.	6	2
Bracon osculator Nees	2	5
Pristomerus sp.	1	2
Gelis tonsus Först	—	1
Other braconids	2	—
Other ichneumons	1	1

In the meantime (November 1955) a further consignment of 5000 parasitised larvae of *C. frischella* had arrived from Europe. The few parasites emerging from them in quarantine (Table 1) failed to parasitise New Zealand material and after some further experimental work were destroyed. After this consignment, work in Europe was discontinued.

The future prospects for biological control appear to be good, but more must be known of the diapause these moths undergo before parasites are imported. Also much more information is required on the life histories and ecology of *C. spissicornis* and *C. alcyonipennella*? in Europe. Even if *frischella* had been one of the coleophorids occurring in New Zealand, the parasitised larvae consigned from Europe would have still been useless for the work as, even after subjection to as much as twelve weeks at 5°c and 10°c in an attempt to terminate diapause, no mass emergences occurred. Indeed only two insects ever emerged from material so treated even after two years when many of the the *Coleophora* larvae were still alive. It can thus be seen that if *C. spissicornis* and *C. alcyonipennella*? have similar life cycles to *C. frischella* (i.e. flight period of four weeks in June-July followed by several months diapause) in Europe there is little hope for control until means of terminating more quickly, or eliminating, the diapause is found.

Methods and Materials

As no work has previously been carried out on methods of distinguishing the caterpillars of the two species of *Coleophora,* critical field studies on their habits could not be undertaken hitherto. Preliminary examination of field-collected material yielded no clues as to main features of difference and so laboratory rearing from determined adults was initiated. Many difficulties were found in attempting to rear the coleophorids in this way. Not the least was the difficulty of having white clover flowering profusely in pots. Flowers were finally obtained at the right time of the year by potting plants from outdoors in nine inch pots during the autumn and placing them in an outdoor insectary; flowers appeared the following spring. Clover grown from seed inside did not flower the first year. Lighting units in indoor insectaries (glass-covered) proved useless in promoting flowering and, in fact, clover grown in these indoor insectaries hardly flowered at all.

All plants used for breeding were kept in open outdoor insectaries (roof and sides covered with clear plastic gauze).

As the larvae feed on developing seeds, it was necessary to ensure that flowers used were pollinated. At first this was done by means of a camel-hair brush but it was later found that introducing honey bees into nylon mesh cages over plants and leaving them there for 24 hours was more convenient and just as sure. After the bees had been removed from the cages, adult moths of each species were caged separately. They were seen to mate and oviposit while in the cages, and final instar larvae of both species were ultimately obtained. It should be noted, however, that very few of the larvae pupated and emerged in some cultures. This was thought to be due to lack of food as the final instar larvae in all cultures were found on new unfertilised flowers on which they were feeding. It would seem, therefore, that bees might well be introduced into the cages at frequent intervals after the initial pollination of the flowers.

The cages used were of nylon mesh on cylindrical steel frames. The nylon being more transparent than muslin enabled one to observe the moths on the flowers and at the same time let more light through to the plants. It had, however, a short life in the outdoor insectaries owing to the action of the sun and moisture. It is believed that "Orlon" would be a more satisfactory material in this respect.

Much work requires to be done on the flowering of white clover and if possible a means of having flowers at any time of the year would be advantageous.

Life Histories

C. alcyonipennella?

This moth is the more common of the two species of coleophorids found in New Zealand. The adults are present from the end of October to the end of March, being most abundant at the end of December and during January. As this is the only coleophorid moth occurring in large numbers at the end of January in Nelson, field populations of active larvae in the heads could be regarded as almost pure populations of *C. alcyonipennella*? and so more is known of the life history of this moth than of *C. spissicornis.*

The adult moths feed on the nectar of white clover. They copulate on the white-clover heads and may remain *in copulo* for many hours.

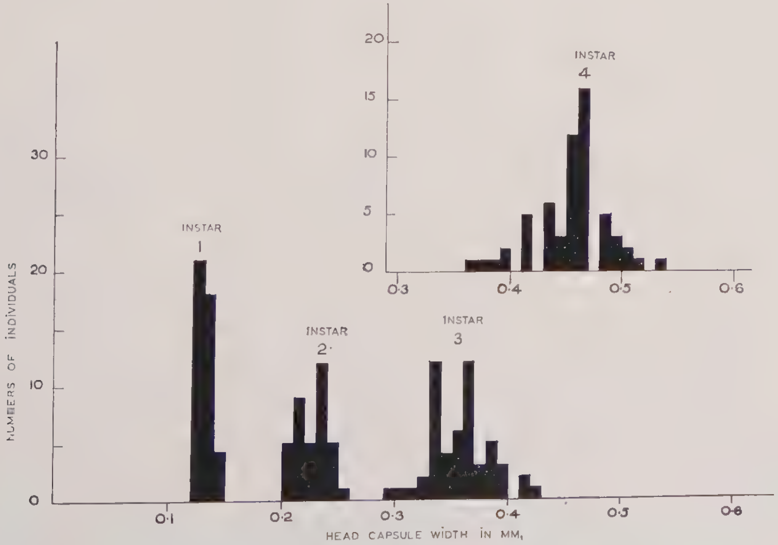


Fig. 1.—Separation of larval instars in *C. alcyonipennella?* by width of head capsule.

During oviposition the female moth inserts its ovipositor between the petals of a clover floret and the calyx and the egg is laid there. The egg is small, approximately 0.35 mm × 0.25 mm, and is punctate.

Four instars have been found by observation of exuviae and by measuring the head capsule widths of 192 field-collected larvae. The larvae were mounted in Berlese's fluid and measured before distortion of the cuticle took place. As can be seen from the histogram (Fig. 1) the head capsules of the 1st and 2nd instars fall into two quite distinct groups. There is no doubt that the first of these groups represents the 1st instar as head capsules from five unhatched larvae were measured and found to fall into this group. The head capsule measurements of the third and fourth groups overlap somewhat but the two instars concerned can be quite easily distinguished by the fact that the pattern of the thoracic shield of the third instar is reduced and different from that of the final-instar larva. It should be noted that the head capsule measurements were made on field-collected active larvae in late January-February and were assumed to be all *C. alcyonipennella*? as no adults of *spissicornis* had been collected for six weeks previously. On later checking of the final-instar larvae used it was noted that only three specimens of *spissicornis* had been used; these were eliminated from the histogram.

The first-instar larva hatches from the egg and immediately burrows through the petal into the developing seed pod. Here it enters one of the developing seeds and devours it, leaving only the outer shell. At this stage the only evidence of a caterpillar being inside the seed pod is the small puncture surrounded by a very small area of discolouration. The first-instar larva usually consumes one seed before moulting. The second instar usually consumes any of the inside of the first seed remaining and then proceeds to devour the next adjacent seed and perhaps part of the next before moulting.

The third instar consumes any remaining seeds in the pod and will then burrow into the next adjacent floret and begin to eat the seeds in it. At this stage the larva remains in the flower head and has no case. It does, however, construct a "tunnel" through the adjacent florets which it webs together. Up to five florets have been seen webbed together in this way and with the contents of their seed pods eaten out by a third-instar caterpillar.

After consuming most of the contents of about five pods, the third-instar larva moults. The final- or fourth-instar larva at first behaves in much the same way as the third-instar but after consuming a few seeds it commences to construct its case. It does this by webbing together the margins of the standard petal of a floret, which at this stage is now brownish, and detaching it from the rest of the floret by neatly severing it at the end nearest the calyx. This case is later reinforced somewhat and in the stages which will become the overwintering generation it is strongly reinforced and is of the typical cigar shape, the posterior end having a Y-shaped opening as described by Hammer (1937) in *C. spissicornis.*

There may thus be two forms of case, one characteristic of the summer generations and the other of the overwintering generation. It should be noted that both types of case may often be seen at the one time and that the variation is not quite clear-cut.

When the final-instar larva has constructed a case it may move to another flower head or perhaps if there are still sufficient pods on the head it is on it will remain there. It feeds extensively and moves about from floret to floret. When feeding, it webs the entrance of the case to a floret and, after consuming the seeds in the pod, frees itself and moves to another floret. Contrary to Hammer's (1937) observation of *C. spissicornis* in Denmark, the larva does not turn in its case to deposit excrement but remains with its head at the opening, the faeces passing through the posterior valves in the normal way. When the larva is fully fed it may do either of two things. It may attach itself to the withered head it is on, seal its case, turn in its case and pupate, or it may migrate quite a distance before attaching itself to some object and pupating. The latter migration seems more typical of the later generations than the earlier ones, though the larva in the winter generation does not pupate immediately but diapauses during the winter months. No emergences have been obtained from overwintering larvae collected in the field, and it may be that there is a two-year overwintering period. This, however, is not proved, and it may be that the conditions under which the larvae were kept did not favour pupation. Preliminary experiments to terminate diapause in which final-instar overwintering larvae of *C. alcyonipennella*? and *C. spissicornis* were exposed to low temperatures of 5°c and 10°c for two months were inconclusive and no larvae emerged when removed to normal temperatures. It would seem that after overwintering the larvae pupate and give rise to the first adult generation in November.

The period from egg to adult as observed in caged material in the outdoor insectary during December-February was from 48–58 days. From observing the first occurrence of final-instar larvae in the field, it would seem that the first generation in nature takes six weeks, a little shorter time than this. In natural conditions the life cycle for the later summer generation will probably be from four to five weeks. These times of course are approximate being not related to temperature or humidity.

There are probably about four or five generations of moth per year though this will vary somewhat with climatic factors.

The length of adult life of field-collected adults in the insectary was from one to two weeks. However, since the material was field collected, it is probable that the insects are somewhat longer lived than this.

The above life cycle of *C. alcyonipennella*? is restricted to white clover. The moth also attacks strawberry clover (*T. fragiferum*) which flowers later than white clover. The life history on this plant is much the same, but the final-instar larva delays making its case. The case is also different, in that the calyx is used to a large extent, the withered florets on strawberry clover being minute. There being only one seed per pod in strawberry clover, the number of seeds consumed is also different.

C. spissicornis

Rather less is known of this moth owing to its shorter flight period and because of the difficulty of distinguishing its larva from that of *C. alcyonipennella?* in the field.

The adult is present from November to January and occurs in large numbers in Nelson in December. A few stragglers may be seen later than January, but by this time it is vastly outnumbered by *C. alcyonipennella?*

So far as is known, details of the larval stages are probably the same as for *C. alcyonipennella?* The larvae of both species are very similar. It should be noted however that *spissicornis* has been recorded only from *T. repens* in New Zealand. *C. alcyonipennella?*, on the other hand, will attack both *T. repens* and *T. fragiferum.* It is surprising that no cases of red clover being attacked by the caterpillars of *C. spissicornis* are yet known with certainty in New Zealand, as red

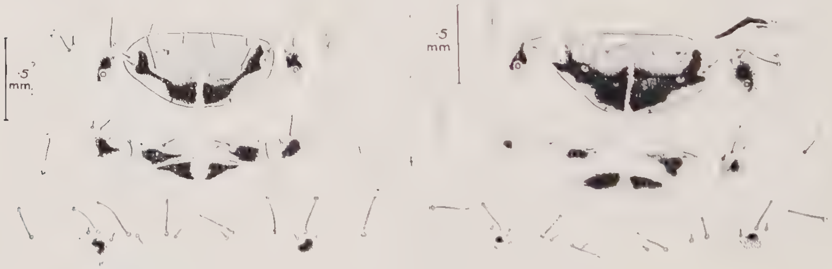


Fig. 2a. Fig. 2b.

Fig. 2.—Pattern of sclerotisation on thorax of final-instar larvae of:
(a) *C. spissicornis*
(b) *C. alcyonipennella?*

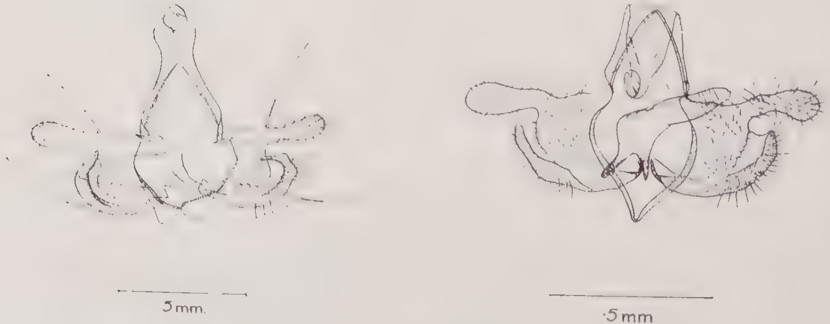


Fig. 3a. Fig. 3b.

Fig. 3.—Male genitalia in:
(a) *C. spissicornis*
(b) *C. alcyonipennella?*

clover would appear to be the main host of *C. spissicornis* in Europe (Hammer 1937).

There appears to be only one generation of *C. spissicornis* per year and the larva diapauses in its case in a similar manner to *C. alcyonipennella*? From observations in breeding cages in an outdoor insectary

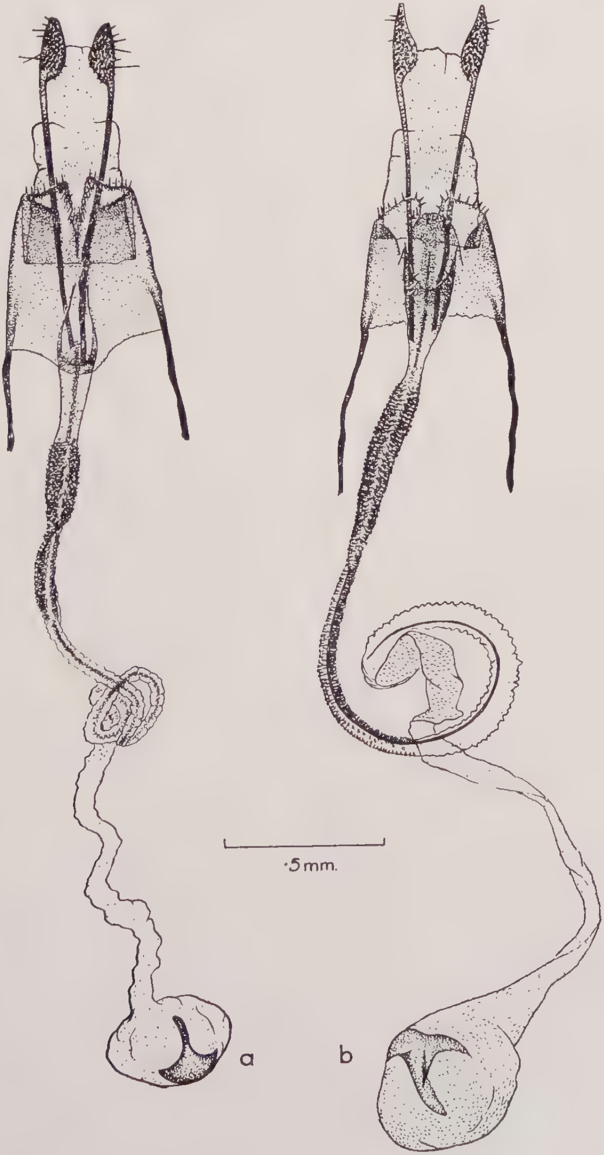


Fig. 4.—Female genitalia in:
(a) *C. spissicornis*
(b) *C. alcyonipennella*?

the life period from egg to adult of *spissicornis* is from five to six weeks, and the length of adult life is approximately two weeks.

The Final-instar Larvae

Both larvae are small, 6 mm long, whitish, and have areas of sclerotisation on the head, thorax, and last segment of the abdomen. General descriptions are given by Hammer (1937) and Hamilton (1944). On examination of the larvae it was seen that there is a distinct and non-variable difference in the pattern of the thoracic shields. In *spissicornis* (Fig. 2a) the area of dark sclerotisation on the prothoracic shield does not extend beyond the β and δ setae of Fracker (1915) and on the median line is confined to the posterior third, while in *alcyonipennella*? (Fig. 2b) the dark sclerotised area extends beyond these setae and extends half way on the median line of the shield; there are also quite clear and contrasting areas surroundings these setae in *alcyonipennella*? As can be seen in the figures there are other minor differences mainly in the pattern of sclerotisation on the mesothoracic shield and in the relative distances between some setae. It is felt however that they are not sufficiently distinct taken separately as characters.

The Adult Genitalia

As some confusion has existed previously, it has been decided to give figures (Figs. 3 and 4) of the genitalia *C. alcyonipennella*? and *C. spissicornis.* Descriptions of both species are given in Dumbleton (1952). It should be again noted that Dumbleton's *frischella* L. is *alcyonipennella*? Kollar. An additional character not previously recorded is seen in the eye colour of the living adults. *C. alcyonipennela?* has white eyes whereas *C. spissicornis* has reddish-maroon ones. No exceptions to this have been seen.

REFERENCES

BRADLEY, J. D. 1956: *Coleophora paripennella* Zeller, 1839, and a Description of a New Species Previously Misidentified (Lep: Coleophoridae). *Ent. Gaz. 7:* 145–9.

DUMBLETON, L. J. 1952: Coleophoridae (Lep.) as Pests of Clovers. *N.Z. J. Sci. Tech. A33(5):* 109–12.

FRACKER, S. B. 1915: The Classification of Lepidopterous Larvae. *Ill. Biol. Monogr. 2* (1): 1–169.

HAMILTON, A. 1944: *Coleophora spissicornis,* Haw., the Clover Casebearer in New Zealand. *N.Z. J. Sci. Tech. A25:* 269-73.

HAMMER, M. 1937: Kloversaekmollete, *Colleophora spissicornis. Tidsskr. Planteavl. 42:* 333–43.

LINDSAY, S. 1927: On the Occurrence in New Zealand of *Coleophora fabriciella* Villers. *N.Z. J. Sci. Tech. 9:* 248.

MEYRICK, F. 1928: "Revised Handbook of British Lepidoptera". 914 pp. Watkins & Doncaster, London.

PIERCE, F. N.; METCALFE, J. W. 1935: "The Genitalia of the Tineid Families of the Lepidoptera of the British Islands." 116 pp. Pierce, Oundle.

SMITH, R. F.; ANDRES, L. 1954: New Pest of Ladino Clover Seed. *Calif. Agric. 8:* 7.

STUDIES IN HOGGET REARING

I. GENERAL CHARACTERISTICS OF HOGGET ILL-THRIFT

By E. A. Clarke and Daisy B. Filmer, Ruakura Animal Research Station, Hamilton.

(Received for publication, 30 September 1957)

Summary

The problem of ill-thrift of hoggets in the autumn in the midst of apparent abundance of feed is discussed and various known causes of unthriftiness are dealt with.

The characteristics and symptoms of the syndrome are given. The most striking symptom is the cessation of growth or loss of weight on well controlled sheep pastures of apparently high nutritive value.

This unthriftiness varies in intensity with year, district, farm, and paddock, but its onset generally coincides with the fresh growth of autumn grass.

Lambs born out of season and weaned at the normal weaning age in early October failed to show ill-thrift in the spring and summer months but ceased to grow during the autumn flush on the same pastures.

It is shown that age and weight at weaning are not factors associated with the development of unthriftiness, nor are breed or sex.

INTRODUCTION

In all sheep-raising countries the problem of rearing young stock through their first year is often a most difficult one. In New Zealand this problem has existed probably from the time sheep were first introduced. Early writings indicate that losses as high as 25% were not uncommonly experienced in young stock. With intensification of farming consequent upon greater sub-division, pasture improvement, top-dressing etc., there has been a more widespread realisation of the comparatively high incidence of losses in young stock. In recent years several specific problems or diseases have been recognised and efficiently controlled, but the overall problem remains.

Marshall (1945) surveying the loss of livestock in New Zealand arrived at an estimated loss of a half million lambs up to 30 April, and 186,000 after 30 April. In addition to losses from mortality of stock there is also a considerable loss accruing from the poor production of animals in unthrifty condition. Although this is difficult to assess, it is obviously of considerable importance. It is made up of lowered meat and wool production and an indirect loss associated with animals failing to reach marketable condition when prices are favourable so that they have to be carried over their first winter in store condition. In spite of the recognition and control of specific diseases, there is still a considerable loss for which no adequate explanation has been made. In particular between weaning time and the end of winter, direct and

N.Z. J. agric. Res. 1; 249–264.

indirect losses in hoggets frequently reach high proportions from causes not at all well defined. This occurs both on fattening and on breeding country.

On the fattening country the aim is to get as many lambs as possible marketed before weaning. Lambs not marketed by this time are either "finished" on pasture or on crops in districts where pasture production slumps or is unsuitable at this time. It is in these hoggets that an ill-defined unthriftiness occurs in the autumn and persists for many months often until late winter. Frequently the ill-thrift results in no more than delayed fattening, but in more extreme cases results in severe loss of weight, emaciation, and a considerable death rate. Purchased store lambs from the hill country often behave in a very similar fashion to these home-bred lambs. Accordingly, many farmers regard the finishing of store lambs on fattening country as something of a gamble, good results being obtained in some years and extremely poor results in others. The dairy farmer who buys in a few store lambs usually achieves very good trouble-free results and not infrequently the view is expressed that the dairy farm is free of "sheep sickness"—a nebulous undefined disease, which, it is presumed, builds up on sheep country. Freedom from trouble in young sheep on the dairy farm has long been taken advantage of by the stud breeder who hopes to develop his ram hoggets into well grown animals by the following summer. On the fattening country, crops are often grown for "finishing" lambs and in general in the drier districts give good results. Often, however, on crop the same lack of thrift in lambs is manifested as on grass.

On the hill country it is common practice for the wether lambs to be disposed of, at or after weaning, as "fats" to the works, and as stores for finishing on the fattening country. The ewe lambs are required for flock replacements and in the rearing of these from weaning to the following spring considerable losses, which follow the same pattern as those on the fattening areas, are not infrequent. In the course of the studies being reported, mortalities in hill-country hoggets of the order of 15% to 25% have been recorded on some farms. The mortality is seldom below 5%, and averages from 5% to 10%. In addition to this direct loss from mortality, there are indirect losses the magnitudes of which are difficult to assess. It has been shown for example by Esplin *et al.* (1940) that in addition to a lowered death rate, lambs well reared over their first winter had heavier fleeces with longer staples and although by breeding time the following autumn the well-reared lambs had lost most of the weight advantage gained over the winter, there were approximately 20% more that had lambs in the well-reared group than in the poorly reared one. Further studies by Phillips *et al.* (1945) showed that lambs well reared in their first winter had better-developed reproductive tracts than the poorly reared lambs. Our results at this Station confirm the finding that well reared lambs give a higher lambing percentage as two-tooth ewes owing to a lower incidence of dry ewes. Also the extra wool production of well reared hoggets has been of the order of 1 to 1.7 lb.

In addition to the higher death rate and lower production of poorly-reared hoggets, there are not enough ewe hoggets to allow selection for flock replacement. Where the lambing percentage in the flock is of the order of 70% and the death rate in the hoggets about 15%, all the hoggets reared to the two-tooth stage are required to keep up numbers in a flock of normal age composition (ewes culled for age at five and a half years). With a lambing percentage of 100 and a death rate in hoggets of only 5%, roughly 25% of the ewe lambs reared would not be required for flock replacements and would be available for sale. This scope for selection is of considerable importance. Even though the productive characters are weakly inherited, the phenotypic selection available is of considerable importance and persists to some degree for the lifetime of the sheep selected (Rae 1947; McMahon 1945).

In New Zealand, although this problem of efficiently rearing hoggets between weaning and the following spring has been widely recognised, comparatively little work has been done to determine its exact nature and causes. Marshall (1945) in his survey, after listing enterotoxaemia, arthritis, and blood poisoning following docking, as specific causes of loss, names parasitic gastro-enteritis as responsible for interference with digestive function, for anaemia, and for loss of condition, with mortalities varying in different seasons and within districts but reaching its highest level in Canterbury. He states that the delayed fattening of many lambs that survive infestation must cause considerable economic loss. A field survey conducted by Ewer and Sinclair (1951) in 1938-39 in Canterbury when losses in sheep were very high showed that the number of worms of pathogenic species carried by unthrifty sheep frequently reached dangerous levels and that the largest number of deaths occurred in lambs just before and for some time after weaning, from December to April. It was at this time that the highest worm counts were obtained. While heavy worm infestation was frequently associated with high mortality, there were indications that there were unknown factors probably associated with the feed itself that were playing a part. In an attempt to elucidate these problems, trials were conducted at the Kirwee Experimental Farm over the years 1940-47. Unfortunately over that period there was no recurrence of the high mortality of the 1938-39 season. There were however small responses in liveweight from drenching in two of the years. From the Canterbury survey and the trials at Kirwee it was concluded that the *Trichostrongylus* spp. were to be found at dangerously high levels when climatic conditions favoured worm multiplication. "It is then that the well-known clinical picture associated with severe worm infestation is seen, a more or less intense unthriftiness associated with lack of appetite, depression, and, in most instances, a persistent diarrhoea, and a desire for water." An experiment conducted by these workers with hoggets on a low plane of nutrition in 1947 suggested "that only where there has been a severe lowering of the plane of nutrition, as well as concurrent favourable weather conditions for worm multiplication, will there be interference with the normal growth rate and a favourable response following drenching."

More recently Salisbury *et al.* (1953) have reported serious mortality in the Marlborough district during the summer of 1951-52 which was investigated conjointly by the workers from the Wallaceville Research Station and the Grahame Veterinary Club. After the elimination of all infective bacterial processes, helminthiasis, and copper and cobalt deficiencies, it was eventually decided that the cause of the trouble was some nutritional factor as yet unknown, complicated by coccidiosis. In this outbreak, feed was abundant as a result of liming and topdressing. The mortality was confined to lambs, and of the 1,490 lambs marked only 370 survived at the end of the winter. Deaths occurring in mid December were thought to be due probably to enterotoxaemia, but from this time onwards the condition of the lamb flock deteriorated rapidly with a high mortality rate. There was inappetance, rapid loss of condition, and evidence of abdominal pain, but no marked anaemia. Scouring was severe, the faeces being watery and yellow brown, and there was marked thirst. The main feature on post-mortem examination was severe emaciation and the hearts were invariably of very poor tone. The only lesions of any significance were a patchy inflammation of the small intestine and a moderately severe haemorrhagic typhlitis. Ruminal contents were very fluid with a strong ammoniacal smell. The intestinal lesions, enteritis and abdominal pain, loss of weight and diarrhoea, the foul-smelling, brown-yellow faeces often streaked with blood, and anaemia sometimes present, are symptoms commonly found in lambs severely affected with coccidiosis. This is not a condition which we have encountered in our studies. Although faecal examinations have been made at Ruakura on many thousands of lambs unthrifty over the autumn period, coccidia oocysts have never been found in significant numbers nor has coccidiosis been reported as a possible cause of ill-thrift and mortality elsewhere in New Zealand.

"Sheep-sickness" is a term commonly applied to pastures on which hogget ill-thrift is manifested during the autumn and winter. The Livestock Division (1930) states that "on low-lying flats with a high carrying capacity the sheep pastures are more likely to become contaminated; the pastures become 'sheep-sick' on account of the absence of cattle". Although not stated, there is implied an association between "sheep-sickness" and parasitism. It is indicated that feed conditions and parasites are inter-related in promoting hogget ill-thrift—mild humid weather resulting in a more or less rank growth of pasture and a more suitable environment for helminth parasites.

Roberts (1933) reports grazing trials in the seasons 1931 and 1932 (conducted at Bangor, north Wales) to compare liveweight increments in lambs run on permanent and on temporary pastures. It was found that newly weaned lambs continued to thrive equally well, at the rate of about 2¼ lb per week, on both pastures until the middle of August. From the middle of August, i.e. early autumn, growth practically ceased in lambs on new pasture and to the end of September averaged 0.6 lb/week for those on new pasture, whereas those on permanent pasture suffered a total loss of 0.5 lb. Roberts states that the failure of lambs to thrive after August on permanent pasture touches a problem affecting many flock masters in north Wales. The lack of thrift varied in intensity and in simple cases the lambs ceased to improve, but often they lost condition and became too thin to be sold even as stores. On some farms in some years the lambs wasted to such extent that many died. He points out that there was no shortage of grass while the lambs lost condition and that mature sheep and growing cattle continued to thrive on the same pastures on which the lambs failed to thrive. He also states that the loss in weight could not be made good at once by removing the lambs to good young pasture—"their health seems to have been fundamentally affected". In discussing possible causes for this loss of condition of lambs on permanent pasture said to be due to their grazing "sheep-sick" land, he indicates that parasitism or a change in the nutritive character of the herbage which affected lambs only might have been causative. While leaving the question open, he indicates that, commonly, lambs that grew badly and even became emaciated in some years on certain good grazing lands were frequently shown on post-mortem examination to be free from parasitic worms. Although, in the trial discussed, more nematode ova were passed in the faeces of lambs on the permanent pasture, he postulates that this may have been the result and not the cause of their condition, since Fraser and Robertson (1933) had shown the effect of nutritional condition on susceptibility to parasitic infestation.

This "sheep sickness" on pasture in the midst of plenty as described by Roberts (1933) bears a striking similarity to the ill-thrift in hoggets seen in New Zealand over the same season of the year.

CHARACTERISTICS AND SYMPTOMS

General

Ill-thrift in hoggets in New Zealand can sometimes be attributed to a specific cause such as mineral deficiency (e.g. cobalt deficiency), haemonchosis, pulmonary infections such as pleurisy and pneumonia, rickets, and starvation due to shortage of feed. Checks in growth of minor intensity and duration are sometimes attributable to diseases such as contagious ophthalmia (pink eye) and contagious ecthyma (scabby mouth). In some seasons facial eczema may be responsible for the failure to thrive, although in its subclinical form this cause is not always obvious.

There is a widespread form of ill-thrift in hoggets for which no obvious cause has been determined. It varies in intensity and incidence from year to year, from district to district, from farm to farm within a district, and sometimes from paddock to paddock within a farm. The symptoms too are often complex and confused. This complexity of symptoms often derives from one or more of the diseases mentioned above which are also present in varying stages and intensity. This ill-thrift results usually in a complete cessation of growth or loss of weight, and may persist so that emaciation results. This starvation in the midst of plenty is the most characteristic symptom of this disease. The animals are generally scouring and are lethargic and difficult to drive. Coughing is sometimes present but this is not associated with the presence of lung worms but invariably with pulmonary infections.

The post-mortem picture is somewhat confused. Characteristically the gut contents are scanty and very watery, and body fat is scant and watery. Anaemia is generally evident and in many cases may be extreme. Bone abnormalities akin to osteomalacia are sometimes quite marked, and fatty infiltration of the liver is generally noted. There is a lack of tone in the muscles generally and in the organs, particularly the heart. In addition, both parasitic and bacterial infections are commonly found.

One of the striking characteristics of this disease is its onset from late summer or early autumn onwards and the spontaneous recovery that occurs usually between May and mid July. In our experience it seldom occurs in the North Island before the end of February and recovery usually commences not later than the end of June. In the 1956 season it would appear to have commenced in January although we believe haemonchosis was in most cases responsible for this early trouble, which was followed by the typical ill-thrift syndrome. The onset of the disease is fairly sudden and in a general way coincides with the autumn flush of pasture. Throughout the dry spell hoggets generally do as well as is consistent with the quantity and quality of feed available. When ill-thrift manifests itself, the hoggets do poorly and virtually starve in the midst of plenty. Recovery is also rapid and spectacular and hoggets will then fatten quickly on the pastures where they previously faded away. The spontaneous recovery from the end of May onwards lends support to the popular belief that once the shortest day is past hogget rearing troubles are over.

Cessation of Live-weight Increase

One of the most striking and invariable symptoms of the ill-thrift syndrome is the failure of the animals to increase in liveweight. Frequently there is a marked loss of weight, which may be of short duration or may persist for over four months. In our experience the affected animals do not lose weight markedly unless severely parasitised or affected by some other specific ailment.

The young growing animal failing to increase in liveweight nevertheless grows, in that changes in body conformation are taking place. Much work has been done on the influence of varying degrees of undernutrition on the growth processes. A trial was carried out by Waters (1908) in which steers were kept on a ration permitting no change in body weight and where the subsequent changes in body conformation were observed. These steers increased in length and height but became exceedingly thin and growth ceased altogether in from 6 months to 1½ years. Their fatty tissue was gradually depleted, finally consisting of only 5% fat and 81% water, but the lean flesh was changed much less in composition and a year of such treatment resulted in nearly 40% loss of lean meat. The skeleton was affected in that the fat was removed from the marrow of the bones and replaced by water. Similar results have been obtained from inanition studies with pigs (Pomeroy 1941).

Lambs on maintenance and sub-maintenance levels of nutrition could, therefore, be expected, after a prolonged period of some months, to show emaciation and starvation symptoms from this cause alone, This is one of the characteristic findings in unthrifty lambs. Post-mortem findings generally disclose further starvation symptoms such as the watery nature of the little fat remaining, depletion of fat in the bone marrow, fatty infiltration of the liver, and scanty and watery gut contents. It is not clear whether bone changes seen, such as an increase in the size of the bone cavity in the long bones or conversely a reduction in the thickness of the bone tissue and a more spongy bone structure particularly at the ends of the long bones and ribs, is entirely attributable to starvation. The poor muscular tone generally and of the heart in particular is likewise a starvation symptom.

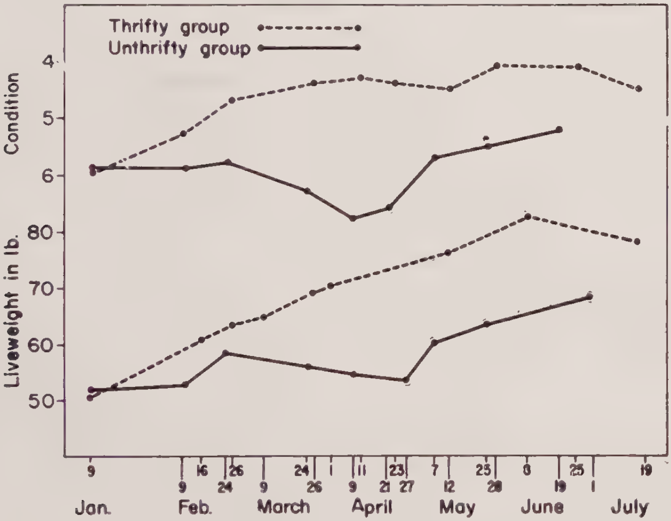


Fig. 1.—Grading and live-weight changes, 1953.

Fig. 1 shows the liveweight changes in two experimental groups of 30 ewe lambs throughout autumn and early winter of 1953. In the same figure, the changes in body grading for fatness are shown. These are subjective gradings which record the tactual impression of state of fatness gained by feeling the back and tail of the animal as is done in the industry in picking fat lambs for killing. Grades are recorded on a scale of 1 (grossly fat) to 10 (extremely emaciated). There is a close correspondence shown in this figure between changes in liveweight and changes in body condition. This indicates clearly how loss of condition rapidly follows changes in body weight and rapidly falls when the animal is showing a maintenance or sub-maintenance live-weight curve.

With few exceptions we have never encountered in our trials ill-thrift as severe as is sometimes seen in the industry where we have observed many animals with a grade estimated as 9. In some cases these animals were heavily parasitised when examined post mortem. The cause of this extreme loss of condition is as yet not clearly understood.

Seasonal Incidence of the Syndrome

That this syndrome does not occur at other times of the year has been demonstrated experimentally over two years. In 1954, by appropriate hormone therapy, ewes were mated so that the lambs were born in the last week of June. Eight lambs were weaned on 12 October at the age of 16 weeks (normal weaning age on to an experimental block at the Ruakura Research Station where for the two previous years hoggets had ben unthrifty ovr the autumn months. These lambs rotationally grazed two 1-acre blocks on the experimental area of twelve 1-acre blocks. Pasture management aimed at keeping the sward at approximately 4 in. in height by the use of mature cattle and by topping with a mower throughout, in the same way as with autumn pastures.

On 11 January 1955, the remainder of this experimental area was stocked with hoggets weaned on that date at an average age of 16 weeks. Fourteen of these hoggets were kept on this area throughout the autumn and early winter and managed in a similar manner to those weaned in the spring, to provide a control group against which the live-weight changes of the spring-weaned lambs may be compared.

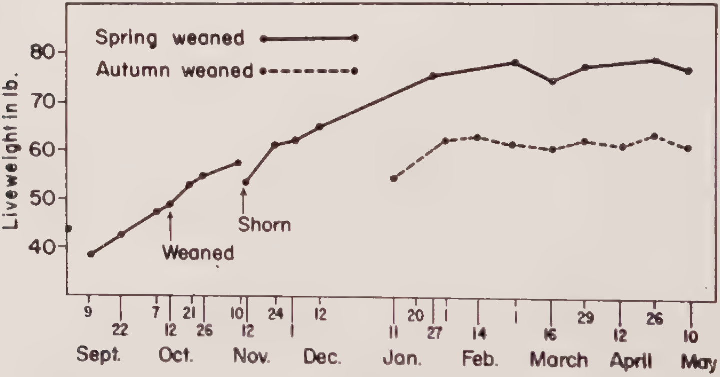


Fig. 2.—Comparison of spring-weaned and autumn-weaned lambs.

The live-weight changes of these two groups of hoggets are shown in Fig. 2. The spring-weaned lambs (when due allowance is made for wool removed at shearing) showed a regular live-weight increase of about 2.2 lb/week between weaning and 27 January 1955. Over the next month, somewhat smaller gains were made, and from 1 March to 10 May they lost 2 lb in weight. The autumn-weaned lambs, although 5½ lb heavier at weaning, showed a striking similarity in their curve of mean live weights, losing about the same amount of weight over the autumn and early winter period. A similar trial, which gave the same results, was carried out in the following year (1955-56) with groups of 13 lambs.

From these two trials it is clear that on this area (which is typical of a large part of the Waikato basin) hogget ill-thrift is confined to the autumn and early winter. This is in line with our general observations and with farmer experience. The onset of ill-thrift in these trials had no relationship to nematode parasite burdens, which were low throughout and comparable with those in thriving hoggets. The role of nematode parasitism in this ill-thrift syndrome will be elaborated in a subsequent section.

Although on the same paddock, the spring and autumn pastures did show botanical differences. Through the summer *Paspalum dilatatum* became more obvious and by the autumn constituted about 20% to 30% of the pasture. In the spring and early summer also, Yorkshire fog was a more conspicuous constituent of the pasture than later. The autumn pastures were not otherwise strikingly different from those in the spring in botanical composition. Furthermore we have observed the occurrence of the typical ill-thrift syndrome on almost every type of pasture to be found over the lowlands and hill country of the North Island. There is no evidence that any change in the botanical composition of the pasture or in the balance of pasture constituents is responsible for the differential response in hoggets weaned in the spring and those weaned in the autumn. Pasture production is far greater in spring and summer than in autumn (Hudson 1933; Clarke *et al.* 1953), but there is little information on the other characteristics of pasture growth at different seasons of the year. In a later paper of this series, it will be shown that, irrespective of pasture composition, the stage of growth of autumn pasture seems to be the factor influencing the thrift of hoggets.

Yearly Variations in Incidence and Intensity

General observation clearly indicates that the incidence and intensity of this problem varies from year to year. The Livestock Division

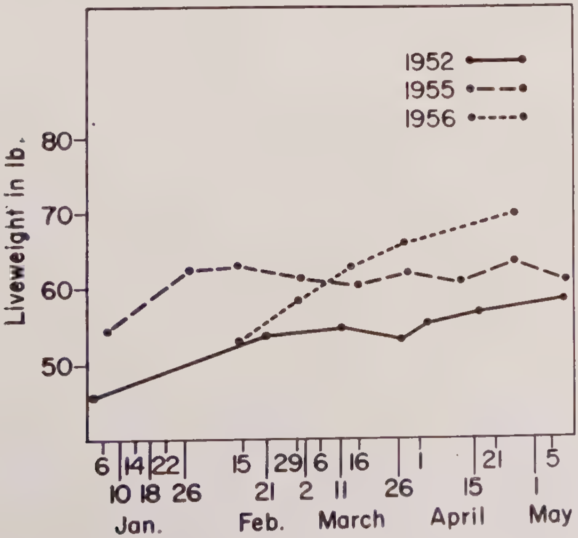


Fig. 3.—Seasonal variations on the same pasture.

(1930) stated that in 1929 and 1930 serious trouble was experienced in both islands in the rearing of hoggets. Ewer and Sinclair (1951) record that considerable difficulty was experienced in the Canterbury district in 1938-39, the condition bearing more than a superficial resemblance to that affecting hoggets in the North Island in the autumn. In the five years that this problem has been under investigation by us, we have observed its incidence to some degree in each year, not only on the Research Station but also on farms over a wide area.

Variations in its intensity and duration in different years have been noted in exxperimental groups under observation. Figure 3 shows the mean live-weight curves of a group of hoggets run through the autumn on the same pastures in three different years. In the 1952 season, there was a cessation of live-weight increase only from 18 February to 26 March, but at no time did the hoggets grow well over the period shown. In the 1955 season, between 1 February and 10 May, the hoggets fluctuated slightly in live weight and showed an overall loss in weight of approximately 2 lb. This is the common picture of ill-thrift in hoggets over the autumn period, although it commenced earlier than usual in that year. In the 1956 season, the hoggets grew throughout with a mean live-weight increase of just under 2 lb per week. Variations of this magnitude are commonly experienced from farm to farm and from year to year.

We believe that these live-weight variations are consequent upon seasonal conditions reflected in pasture growth. In a later paper of this series, it will be shown that grazing management can play a very important part in the onset, duration, and intensity of ill-thrift, irrespective of climatic conditions and pasture growth. Our data suggest that varying combinations of climatic conditions reflected in pasture growth and grazing management are responsible for the variations seen in the onset, intensity, and duration of ill-thrift in hoggets from year to year, and from district to district.

Age and Weight

That loss of weight on autumn pastures is not confined to newly weaned hoggets was clear from the trial where lambs were weaned in the spring (Fig. 1). In the 1954 season, a few two-tooth wethers were run with each of two experimental groups of hoggets on grass. In both cases a similar result was obtained and this is illustrated in Figs. 4 and 5 which show the mean live-weight changes in groups of 7 two-tooth wethers and of about 40 ewe lambs in each trial. The similarity of the live-weight curves of the hoggets and of the two-tooths is clear and the magnitude of the live-weight changes, irrespective of age, is particularly striking. Relative to live weight, however, these changes are twice as great in the hoggets as in the two-tooth wethers.

Loss of live weight in the autumn in mature breeding ewes in the Manawatu district over seven years under *ad lib.* pasture feeding has previously been clearly demonstrated (Clarke *et al.* 1953).

These data indicate that the check in live weight in the autumn is not due to some factor intrinsic in the hogget. In the mature animal, probably because live weight loss is relatively small, the typical ill-thrift so frequently seen in the hogget would seem not to develop. Furthermore, in the older animal a resistance to diseases, especially parasitism, has been developed.

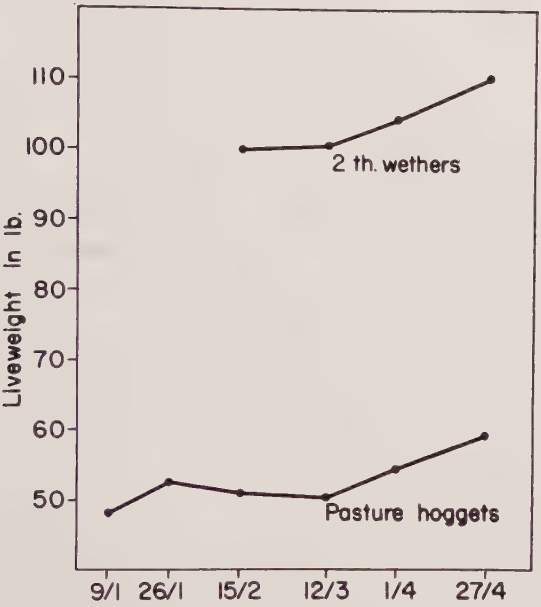


Fig. 4.—Comparison of 2-tooth wethers and hoggets.

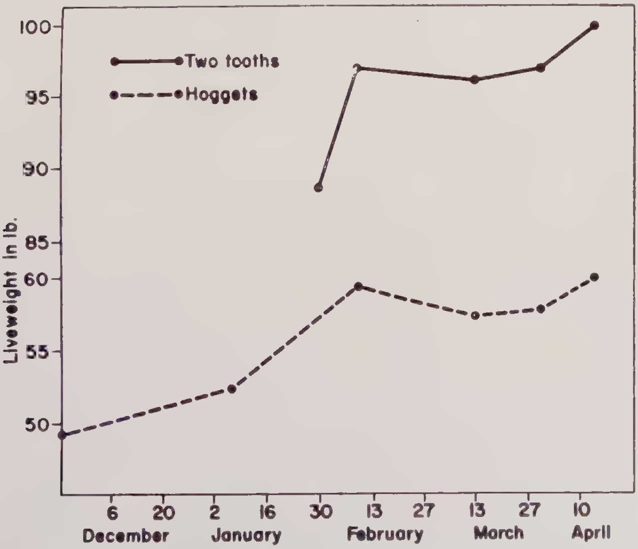


Fig. 5.—Comparison of 2-tooth wethers and hoggets.

It is popularly believed that older lambs and bigger lambs at weaning are less subject to the ill-thrift syndrome. The Livestock Division (1930) makes this point and states the need for greater attention to the feeding of the culls left by the fat-lamb picker. The fact that these "tail-enders" are commonly the ones that are carried through the autumn and early winter—a period difficult in any case for lambs perpetuates the widespread belief that late lambs and small lambs are the ones most subject to ill-thrift.

In our trials over five years, the experimental flocks have been random groups covering the entire range within our lamb crop for age, weight, and condition at weaning. Our data do not support the belief that young lambs and small lambs including many of the twins are more susceptible. When a "tail end" (or a small group of markedly unthrifty lambs) does develop, it is composed of lambs varying widely in age and size. It is our experience that the ill-thrift is manifest throughout the flock generally. Fig. 6 shows the mean live-weight curve of approximately 50 lambs comprising unculled ewe lambs from an experimental flock. These lambs were all weaned on 20 December 1955 and run together on pasture throughout. Nematode parasites were kept at negligible levels (as indicated by faecal egg-counts) by drenching with doses of 20 g phenothiazine at approximately two-weekly intervals throughout. Fig. 6 also shows the live-weight curves of the largest and smallest hoggets at weaning, and these are very similar to the group mean.

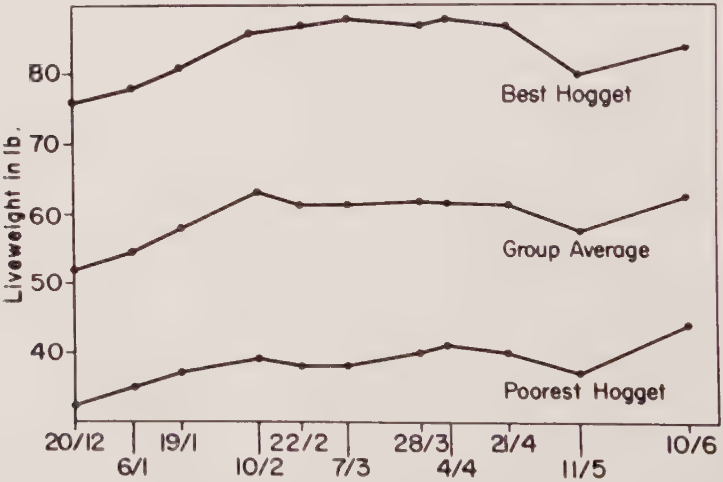


Fig. 6.—Effect of condition of lambs.

Commencing on 10 February 1955, the hoggets ceased to increase in live weight and between this date and 11 May 1955 an average loss of about 6 lb was recorded. This fairly sudden cessation of live-weight increase persisting over a period of three months or more and showing a fairly sudden recovery thereafter is a common picture of ill-thrift in lambs in which parasitism is controlled by regular and frequent

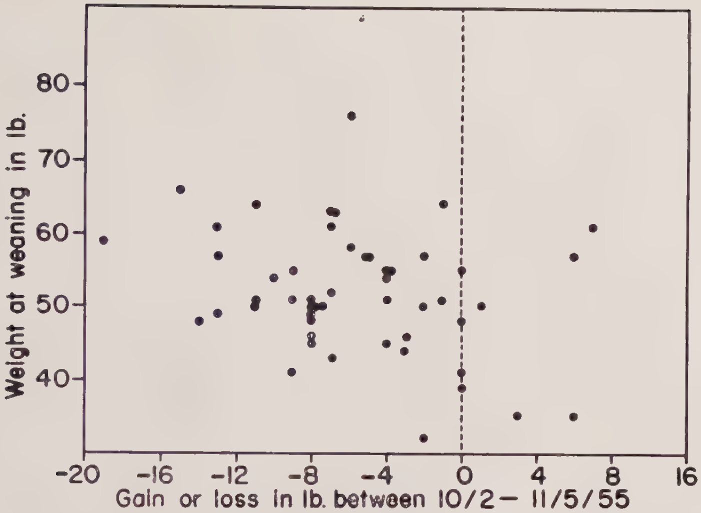


Fig. 7.—Relation of weight at weaning to live-weight changes.

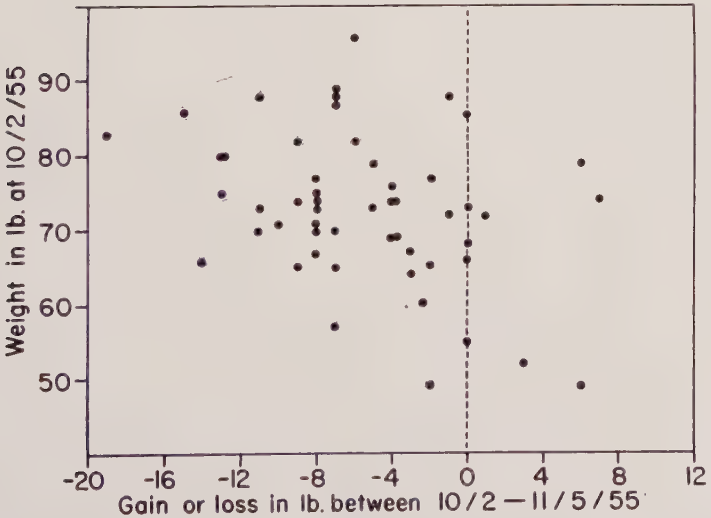


Fig. 8.—Relation of weight at start of ill-thrift period to live-weight changes.

drenching. There were no deaths over this period although many individual lambs showed quite large losses in live weight (Figs. 7 and 8).

Fig. 7 shows the lack of any marked correlation between weaning weight and live-weight change over the period when ill-thrift was manifest. Some of the lighter lambs over this period show only small losses or a slight gain. The greatest loss was shown by a lamb slightly above average weight at weaning.

In Fig. 8 the changes in live weight in the lambs are plotted according to their weight at the commencement of the ill-thrift period. As in the previous figure, it is clear that the lightest lambs are not necessarily the most severely affected.

Similarly, age at weaning shows no correlation with loss in weight over the ill-thrift period (Fig. 9). Age at weaning varies considerably from 77 to 122 days. Some of the youngest lambs have shown little or no loss in weight over the ill-thrift period, whereas some of the older lambs show a considerable loss.

Although twin lambs do not occur frequently in our hill country flocks, our data show clearly that the same individuality exists within these as within single lambs in their live-weight changes through a period of hogget ill-thrift.

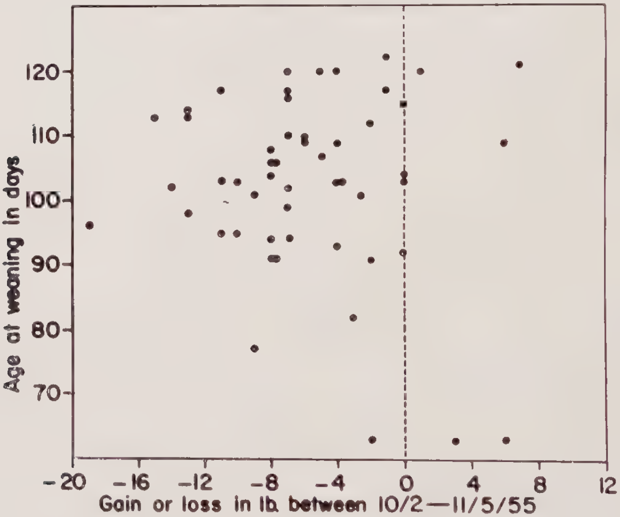


Fig. 9.—Relation of age at weaning to live-weight changes.

Breed

Ill-thrift in the autumn is observed in hoggets of various breeds. It is frequently seen in Down-cross lambs not "finished" off the mothers, in a form as severe as in hill-country white-face lambs. Early in this century when the Lincoln breed was commonly used on hill country, ill-thrift is said to have been very severe and largely responsible for the rapid decline of this breed. There are, however, no data except reports of heavy hogget mortalities in this breed to support this belief. Over the past four years we have been able to compare the thrift of Romney and Border Leicester × Romney half-breds run together on pasture.

The results shown graphically in Fig. 10 for 20 half-breds and 15 Romneys run together throughout on pasture in the 1953 season illustrate the breed comparison. Results for the other years entirely

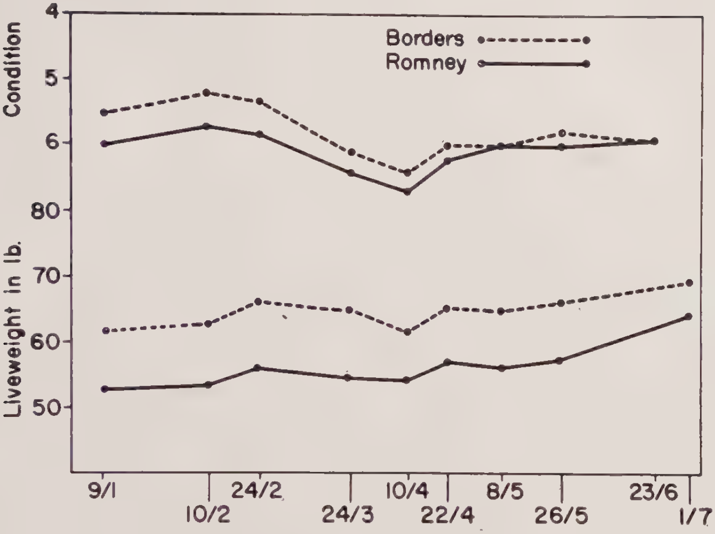


Fig. 10.—Effect of breed.

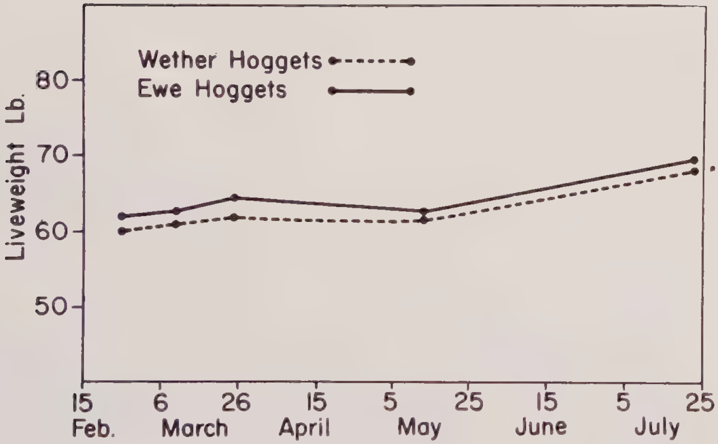


Fig. 11.—Effect of sex.

confirm the results shown in this figure. In this year a loss of weight and a marked loss of condition occurred over the autumn period, both groups reacting to the same extent and in a similar manner. The heavier weight at weaning of the half-bred lambs has been noted in each year and tends to be maintained throughout the first winter and thereafter increases. The remarkable parallelism of the two curves has each year been a striking feature of this breed comparison. It is quite clear that, in spite of the greater initial size of the half-bred lambs and their greater vigour as evidenced by their more rapid gain in weight to weaning, they were just as susceptible to and equally affected by checks over the autumn period. Nematode parasitism was kept at a low level in both groups by frequent drenching. The difference between breeds is of the same nature as that between hoggets of different ages in Figs. 4 and 5.

Sex

It is common experience that ewe and wether lambs alike are equally affected by ill-thrift over the autumn and early winter. Fig. 11 shows the mean live-weight changes of about 60 ewe lambs and a group of eight wether lambs running with them throughout. Again, the close similarity of the curves is very striking. Each year in our hogget trials a small group of wether hoggets has been run with each group of experimental ewe hoggets and in every case a result similar to that shown in Fig. 11 has been obtained. We have no similar data for ram hoggets, but our experience with rearing Romney ram hoggets through the autumn is that they can be as badly affected as ewe hoggets. Stud breeders overcome this problem in various ways, such as stocking their ram hoggets lightly on dairy-farm pasture, on new white-clover-dominant swards, on crops, by provision of concentrates. With such stock, the cost of rearing is a comparatively small item to set against the market value of the well-grown two-tooth ram.

REFERENCES

CLARKE, E. A.; BARTON, R. A.; WILSON, G. S. 1953: "The Effect of Highly Improved and Topdressed Pastures on the Thrift and Production of Sheep". Massey Agricultural College and N.Z. Department of Scientific and Industrial Research.

ESPLIN, A. C.; MADSEN, MILTON A.; PHILLIPS, RALPH W. 1940: Effects of Feeding Ewe Lambs During their First Winter. *Utah agric. Exp. Sta. Bull. 292.*

EWER, T. K.; SINCLAIR, D. P. 1951: Internal Parasitism in Canterbury Sheep. *N.Z. J. Sci. Tech. A32 (5):* 35—48.

FRASER, D.; ROBERTSON, A. H. H. 1933: *Nature, Lond. 131:* 94.

HUDSON, A. W. 1933: *N.Z. Dep. sci. industr. Res. Bull. 31.*

LIVESTOCK DIVISION 1930: *N.Z. J. Agric. 40:* 38–40.

MCMAHON, P. R. 1945: *Wool Res. Spec. Rep. 1, Wool Tech. Lab. Canterbury Agric. Coll., Lincoln.*

MARSHALL, D. 1945: *Proc. 5th annu. Conf. N.Z. Soc. Anim. Prod.*

PHILLIPS, R. W.; MCKENZIE, F. F.; CHRISTENSEN, J. V.; RICHARDS, G. S.; PETERSON, W. K. 1945: Sexual Development of Range Ewe Lambs as Affected by Winter Feeding. *J. Anim. Sci. 4:* 342–6.

POMEROY, R. W. 1941: *J. Agric. Sci. 31:* 50–73.

RAE, A. L. 1947: Present-day Knowledge of the Inheritance of Productive Qualities in Sheep and its Practical Application in Sheep Improvement. *Proc. 10th annu. Meet. Sheep Fmrs., Massey Agric. Coll.*

ROBERTS, E. J. 1933: *J. Minist. Agric. 40 (4).*

SALISBURY, R. M.; MUIR, J.; STIRLING, J. 1953: *N.Z. Vet. Rec. 1 (4).*

WATERS, H. J. 1908: *Proc. Soc. Prom. agric. Sci. N.Y.*

EELWORM (*Anguina agrostis* (Steinb.) Filipjer) IN *Agrostis tenuis* Sibth. IN NEW ZEALAND

By A. J. Healy, Botany Division Department of Scientific and Industrial Research, Christchurch

(*Received for publication, 15 October 1957*)

In January 1955, plants of browntop (*Agrostis tenuis* Sibth.) near Sheffield, Canterbury, were found to have malformed heads, subsequent examination of which showed eelworm galls in the spikelets.

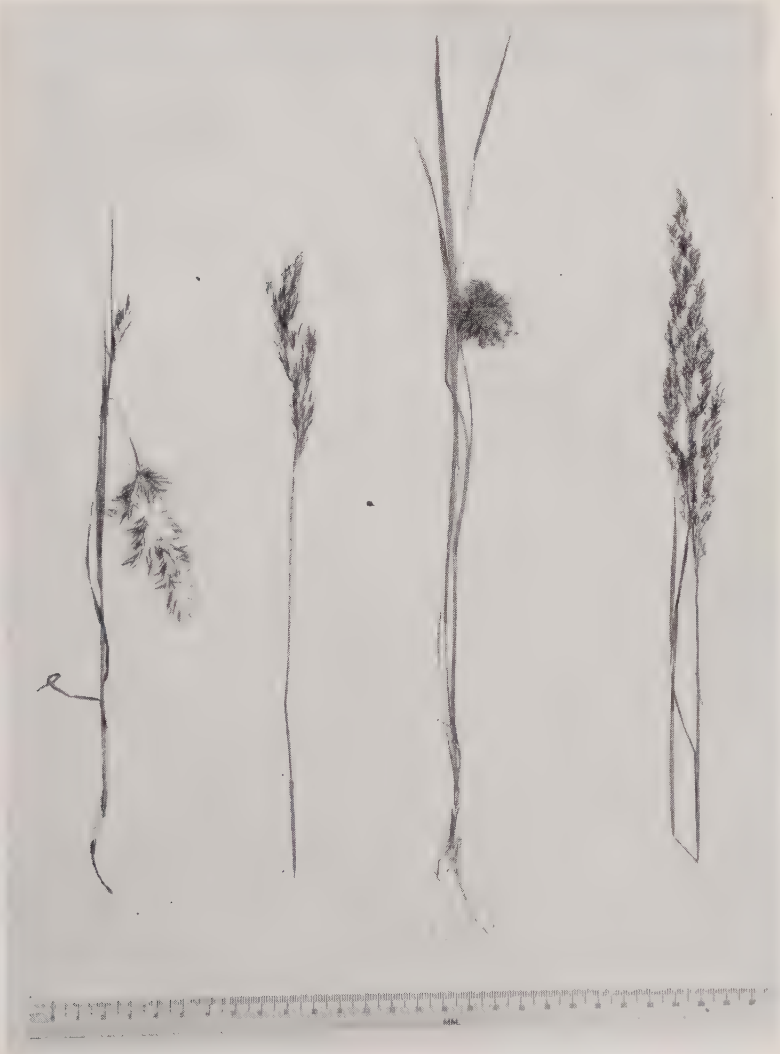


Fig. 1.—Inflorescences of *Agrostis tenuis* Sibth. showing malformation.
—Photo. N. S. Beatus

N.Z. J. agric. Res. 1; 265–266.

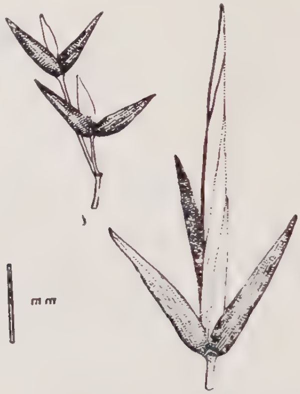


Fig. 2.—Spikelets of *Agrostis tenuis* Sibth. Left—normal spikelets. Right—malformed spikelets showing elongation of glumes and lemma, and gall protruding.

—Drawing, N. M. Adams.

The affected inflorescences showed varying degrees of malformation ranging from occasional spikelets only to every spikelet involved, typical examples being shown in Fig. 1. Affected spikelets show elongation of the glumes and lemmas with replacement of the ovary by a spindle-shaped purplish gall (Fig. 2).

W. R. Philipson in his monograph of the British species of *Agrostis* (J. Linn. Soc. (Bot.) 51: 75-151) noted *Anguillulina agrostis* (Steinb.) Goodey as parasitic on species of the group, and it was considered that this New Zealand material probably belonged to the same species. This tentative determination was subsequently confirmed by Dr. Jones, with, however, a different valid name.

Distribution and Significance

In the summer of 1954–55, infestation appeared to be very severe in the Sheffield, Springfield, Coalgate, and Darfield localities, while in the following summer the incidence of infestation over the same area appeared to be only slight, indicating considerable seasonal variation.

Since browntop seed is harvested in the localities in question, it would seem that in some seasons at least there could be significant reduction in yield of seed owing to eelworm attack. No such loss seems to have been detected, although Dr. Cottier advises that he recalls infestations about 1937.

Acknowledgements

My thanks are due to Mr. P. L. Thomas, Animal Research Station, Wallaceville, for transmission of infested material to Rothamsted, Dr. F. S. W. Jones, Nematology Section, Rothamsted Experimental Station, England, for confirmation of the tentative identification, and Dr. W. Cottier, Entomology Division, Nelson, for information of the earlier occurrence of the eelworm.

EFFECTS OF INGESTION OF PARAFFINS BY RUMINANTS

IV.* EFFECTS OF PARAFFINS AND OTHER SUBSTANCES ON THE UTILISATION OF HIGH DOSAGE LEVELS OF CAROTENE

By W. A. McGillivray and N. A. Worker, Massey Agricultural College, Palmerston North

(Received for publication, 21 November 1957)

Summary

The effect of various paraffins and other non-digestible or poorly absorbed materials on the utilisation of carotene has been further investigated. Paraffins, waxes, and a sterol were found to depress carotene utilisation, the effect being related to the concentration present in the vehicle. The depressant effect decreased with increasing melting point. The triglyceride composition of the vehicle was also found to be important, carotene being less efficiently utilised from the softest and hardest fats investigated.

Introduction

In previous papers in this series (McDowall *et al.* 1957; McGillivray 1957a) it has been shown that the drenching of pasture-fed cows with paraffin oil, for the control of bloat, has a marked effect on the utilisation of carotene as indicated by decreased levels of blood carotene (and to some extent vitamin A ester) and milk-fat carotene and vitamin A. Reid (1957) has shown that of the grades of paraffin commercially available, medicinal paraffins of B.P. quality are the most suitable for bloat control because they have little effect on the feed intake of the animals. In view of the results obtained with this grade of oil and the postulated explanation for its effect on carotene utilisation, it seemed of interest to investigate the effects of other paraffin fractions and of other relatively non-digestible substances such as waxes and sterols. In view of the results obtained, the experiment was extended to include an investigation of the effect of the triglyceride composition of the fat used as a vehicle for the carotene.

Experimental

Chickens, partially deficient in vitamin A, were used in this investigation, and the technique employed was similar to that routinely followed in this laboratory when using rats as experimental animals. The chickens were obtained as day-old cockerels from the College Poultry Farm and were raised in a brooder on a commercial chick mash† which had been found to contain no vitamin A and to be virtually carotene free. After about six weeks on this diet the birds were a convenient size to handle and were almost completely deficient in vitamin A as indicated by extremely low liver reserves.

---

* Previous papers in this series were published in *N.Z. J. Sci. Tech. Sec. A.*

† Supplied by Farm Products Co-operative, Manawatu Ltd., Palmerston North.

N.Z. J. agric. Res. 1: 267–272.

β-carotene* was dissolved in di-ethyl ether and appropriate quantities added to mixtures of olive oil (B.P. quality) and other ingredients to give, after removal of the ether under vacuum on a waterbath, solutions containing 4 mg carotene per gramme of oily material. One-gramme quantities of each of these solutions were then mixed with approximately 5 g of the mash and fed to individual chickens. Prior starvation for about 24 hours ensured that the whole of the prepared mash was eaten rapidly. The birds were then fed normally until slaughter 48 hours after receiving the carotene. Livers were removed and assayed for carotenoids and vitamin A using the methods described by Thompson *et al.* (1949) and McGillivray and Worker (1957).

TABLE 1. Effect of Addition of Various Substances to Olive Oil on Liver Stores of Vitamin A and Carotenoids 48 Hours After a Single Oral Dose of 4 mg/β-carotene and 1 mg α-tocopheryl Acetate in 1 g Oily Material
(Each result represents the average of three livers)

Substance	%	Liver		
			Vitamin A	
		Carotene µg	Alcohol µg	Ester µg
Controls	—	—	0.6	0.6
Olive oil alone	—	12	1.8	84
Paraffin oil	10	18	1.5	23
	30	11	1.4	21
	50	11	0.7	16
Petroleum jelly	10	18	1.3	71
	30	15	1.1	53
	50	11	1.1	34
Paraffin wax	10	24	1.7	97
	30	17	2.5	86
	50	12	1.4	60
Lanolin	10	13	1.2	44
	30	12	1.0	25
	50	13	1.1	19
Beeswax	10	17	2.1	94
	30	16	1.9	81
	50	21	0.8	67
Ergosterol	10	28	2.2	100
	30	10	1.1	79
	50	13	2.4	60
Tributyrin	10	19	3.3	86
	30	15	2.8	71
	50	12	3.0	45

---

* Obtainable from L. Light & Co.

RESULTS

From the results presented in Table 1, it is clear that increasing concentrations of all the substances investigated decreased the utilisation of carotene as indicated by liver stores of vitamin A. In the case of related materials, such as the paraffins and the two waxes, the lower-melting substances produced the greater depressions in carotene utilisation. Although somewhat variable, the levels of carotene in the livers were related, in general, to the amount of vitamin A present.

From Table 2 it is apparent that the triglyceride composition of the fat used as a vehicle for the carotene can also markedly affect utilisation. Liver stores of vitamin A increased as the proportion of tristearin in the fat was increased to 30–40%, but higher proportions of tristearin caused a reduction in liver vitamin A storage.

TABLE 2. EFFECT OF TRIGLYCERIDE COMPOSITION OF VEHICLE ON LIVER STORES OF VITAMIN A AND CAROTENOIDS 48 HOURS AFTER A SINGLE ORAL DOSE OF 4 mg/β-CAROTENE AND 1 mg α-TOCOPHERYL ACETATE IN 1 g OIL

(Each result represents the average of three livers)

Approximate Composition of Vehicle		Liver		
			Vitamin A	
Olive Oil %	Tristearin %	Carotene μg	Alcohol μg	Ester μg
Controls		0	0.6	0.6
100	0	12	1.8	84
90	10	22	1.7	86
80	20	14	1.6	97
70	30	15	1.7	103
60	40	13	1.9	102
50	50	17	1.9	98
40	60	14	1.8	92
30	70	24	2.0	95
20	80	14	2.1	91
10	90	18	1.8	85
0	100	19	1.5	73

DISCUSSION

It is well recognised that the quality of the fat in which carotene is dissolved has a marked effect on utilisation of the provitamin. Early workers in this field (Dyer *et al.* 1934) showed that carotene was 5–6 times more potent for rats when dissolved in arachis oil than when dissolved in hardened cotton-seed oil. In general, solutions of carotene in arachis oil and coconut oil were equally effective, but Lathbury and Greenwood (1934) found, between different samples of these oils, variations in effectiveness as vehicles for carotene, which could not be related to factors such as state of oxidation of the oil. Typical of a large number of later reports is the work of Lease *et al.* (1939) who found that carotene dissolved in cotton-seed oil, decolourised butterfat, soya-bean oil, lard, coocnut oil, or crude peanut oil was equally well utilised but was less effective in triolein, linseed oil, and refined peanut oil.

At about this time interest was centring in the role of antioxidants and the influence of substances like tocopherol on the utilisation of carotene, and since antagonism between unsaturated fatty acids and tocopherol (Sherman 1941) afforded an explanation for some of the differences between the fats, this tended to overshadow consideration of other aspects of fat quality. That other factors can markedly influence carotene utilisation is however apparent from work such as that of Brown and Bloor (1945) who investigated various butterfat fractions. The non-volatile liquid fractions were well absorbed and resulted in the highest liver stores of vitamin A; the poorly absorbed solid fractions were associated with lower liver stores, and the poorest storage resulted from the inclusion of the "volatile" fraction. These results are similar to those obtained in the present investigation where increasing the hardness of the fat above an optimum level was found to reduce the utilisation of the carotene contained in it. At the same time too soft a fat, or the inclusion of tributyrin, also resulted in impaired carotene utilisation. Since a reasonable level of tocopheryl acetate was fed with the carotene, it seems unlikely that variations in the natural tocopherol content of the vehicles would have been of any significance. The impaired utilisation of carotene from the higher-melting triglyceride mixtures is probably merely a reflection of poor fat absorption, a clear inverse relation having been established by a number of workers between melting point and digestibility of fats melting above 50°c (see review by Deuel, 1955). The effect of tributyrin and the observation that carotene is also less well utilised from very soft fat samples is more difficult to explain. The possible injurious nature of a triglyceride like tributyrin cannot be overlooked. At the same time it is clear that factors other than melting point influence fat digestibility. For example mutton tallow (m.p. 47°c) has a higher coefficient of digestibility than cacao butter (m.p. 28°c) while cacao butter, butterfat, and coconut oil, each melting below body temperature, have widely different coefficients of digestibility (Hoagland and Snider, 1943). The impaired utilisation of carotene from the softer triglyceride investigated may therefore also be related to the digestibilities of the fats.

From the results presented in Table 1, it is clear that a number of non-digestible substances depress carotene utilisation. Confirming previous reports (see review by McDowall *et al.* 1957), paraffin oil was found to have a marked effect, the inclusion of as little as 10% in the vehicle reducing liver storage of vitamin A to about one quarter. The other hydro-carbons investigated also depressed utilisation but to a lesser extent, the effect, at each dosage level, decreasing through the series paraffin oil, petroleum jelly, paraffin wax. A similar trend is apparent in the case of the waxes, the harder beeswax having less effect than the softer lanolin. The inclusion of non-digestible or poorly absorbed substances in a fat may influence its digestibility, but in the case of carotene the more important factor is the partition of the carotene between the digested triglycerides and the undigested residue (McDowall *et al.* 1957). This partition is apparently strongly in favour of the undigested residue in the cases of paraffin oil and lanolin but less so with petroleum jelly, paraffin wax, beeswax, and ergosterol. It might be expected that the solubility of carotene in these latter substances would be relatively low and their effect on its utilisation correspondingly less.

From the results presented here, it is apparent that the nature of the vehicle has a marked effect on the uptake and utilisation of carotene. So far as cattle are concerned, the effect of paraffin oil, used for the control of bloat, has already been discussed (McDowall *et al.* 1957; McGillivray 1957a). It seems probable that higher-melting hydrocarbons would have less effect on carotene uptake, but preliminary experiments with paraffin-wax emulsions suggest that these fractions are not as effective as paraffin oil for bloat control (Reid and Johns 1957). It is certain, however, that the utilisation of carotene from pasture will be influenced by the nature of the fatty material present. Plant waxes and sterols would be expected to depress utilisation which would also be influenced by the triglyceride composition of the fat. Seasonal or stage-of-growth variations in the nature and proportion of plant waxes, sterols, and triglycerides might offer an explanation for the abnormal seasonal variations in carotene utilisation as indicated by carotene and vitamin A ester blood levels (McGillivray 1957b) and carotene and vitamin A in the milk fat (McDowell and McDowall 1953). Changes in triglyceride composition of the fat due to microbial hydrogenation during passage through the rumen may also be important.

ACKNOWLEDGEMENTS

The authors are indebted to the Department of Scientific and Industrial Research for a grant towards the investigation and to Mr. J. H. Kissling of the Massey College Poultry Farm for the supply of chickens.

REFERENCES

Brown, E. F.; Bloor, W. R. 1945: *J. Nutr. 29:* 349.

Deuel, H. J. Jr. 1955: "The Lipids." Vol. 2, pp. 215-30. Interscience Publishers, New York.

Dyer, F. J.; Key, K. M.; Coward, K. H. 1934: *Biochem. J. 28:* 875.

Hoagland, R.; Snider, G. G. 1943: *J. Nutr. 25:* 295.

Lathbury, K. C.; Greenwood, G. N. 1934: *Biochem. J. 28:* 1665.

Lease, E. J.; Lease, J. G.; Steenbock, H.; Baumann, C. A. 1939: *J. Nutr. 17:* 91.

McDOWALL, F. H.; McGILLIVRAY, W. A.; REID, C. S. W. 1957: *N.Z. J. Sci. Tech. A38:* 839.

McDOWELL, A. K. R.; McDOWALL, F. H. 1953: *J. Dairy Res. 20:* 76.

McGILLIVRAY, W. A. 1957a: *N.Z. J. Sci. Tech. A38:* 878.

——————1957b: *J. Dairy Res. 24:* 95.

McGILLIVRAY, W. A.; WORKER, N. A. 1957: *Brit. J. Nutr. 11:* 47.

REID, C. S. W. 1957: *N.Z. J. Sci. Tech. A38:* 825.

REID, C. S. W.; JOHNS, A. T. 1957: *N.Z. J. Sci. Tech. A38:* 908

SHERMAN, W. C. 1941: *Proc. Soc. exp. Biol. N.Y. 47:* 199.

THOMPSON, S. Y.; GANGULY, J.; KON, S. K. 1949: *Brit. J. Nutr. 3:* 50.

THE EFFECT OF TOCOPHEROL ON THE UTILISATION OF INTRAVENOUSLY ADMINISTERED CAROTENE

By W. A. McGillivray and N. A. Worker, Massey Agricultural College, Palmerston North

(Received for publication, 1 November 1957)

Summary

The effect of tocopherol on the conversion of intravenously administered aqueous dispersions of carotene in Tween 40 (polyoxyethylene-sorbitan monopalmitate) has been further investigated. High levels of tocopherol in the blood and tissues have been found to inhibit the conversion of carotene to vitamin A and on the basis of results obtained with preliminary, simultaneous, and subsequent injections of tocopherol, it is concluded that intravenously administered carotene undergoes a rapid breakdown, possibly in the blood, to an intermediate which is then more slowly converted to vitamin A.

INTRODUCTION

The effect of tocopherol on the utilisation of orally administered carotene has been fully investigated by a number of workers (see recent review by Hebert and Morgan 1953). It is well recognised that, while small supplements of tocopherol enhance the utilisation of carotene, larger quantities inhibit its conversion to vitamin A. Oral administration does not provide a means of further investigating the mechanism of this tocopherol inhibition which may be merely an effect on the stability of the carotene in the intestine, and possibly on its absorption, or may be a more specific effect on the enzyme systems involved in the conversion of carotene to vitamin A. It has been shown however (e.g. Bieri and Sandman 1951; Bieri and Pollard 1954; Kon *et al.* 1955; McGillivray *et al.* 1956; McGillivray and Worker 1957) that suitable aqueous dispersions of carotene are relatively well utilised by rats when injected intravenously. This mode of administration enables the possible effects, on the mechanism of the conversion of carotene to vitamin A, of substances like tocopherol to be studied without the complicating factors of intestinal stability and absorption.

In experiments of this type Bieri (1955) found that the dosing of ally with tocopherol prior to carotene injection had no effect on of utilisation of carotene as determined by growth and n A studies. The levels of tocopherol obtained in the tissues were however relatively low. Using considerably higher levels of tocopherol injected simultaneously with the carotene, McGillivray and Worker (1957) found a marked depression in liver vitamin A stores as the amount of tocopherol was increased. One milligramme of α-tocopheryl acetate reduced the liver stores of vitamin A derived from injections of 400μg of aqueously dispersed β-carotene by about 25% while 100 mg resulted in a 75% reduction. Since tocopherol has such a marked effect on carotene utilisation, it seemed that experiments in

N.Z. J. agric. Res. 1: 273 280.

which the fate of injected tocopherol was followed, and in which carotene was injected at various time intervals before and after tocopherol injections, might throw further light on the site and rate of the conversion of intravenously administered carotene to vitamin A.

EXPERIMENTAL

All rats used were albinos of the Wistar strain. For vitamin A work a basal diet* of the following percentage composition was employed: ground wheat 42; ground barley 10.5; ground oats 4; dried skim milk 34; wheat germ 8: $CaCO_3$ 1; NaCl 0.5. Pregnant does received this ration throughout gestation and subsequent lactation. The young continued to receive the diet after weaning, and at slaughter (200–300 g) were almost completely deficient in vitamin A as evidenced by low levels of vitamin A in the blood and only traces in the liver.

Aqueous dispersions of β-carotene and α-tocopheryl acetate* were used for injection. These were prepared with a 20% (v/v) solution of Tween 40† (polyoxyethylene-sorbitan monopalmitate) as described by Bieri and Pollard (1954). Di-ethyl ether anæsthesia was used and all injections were into the iliac vein exposed by ventral leg incision.

Carotene and vitamin A were estimated in individual blood and tissue samples essentially as described by Thompson *et al.* (1949) and McGillivray and Worker (1957). Tocopherol was determined using the method described by Worker (1957a).

RESULTS

Injection of Tocopherol Alone

The results presented in Table 1 show that, following the intravenous injection of an aqueous dispersion of 20 mg α-tocopheryl acetate, there was a rapid disappearance of this substance from the blood. After 5 minutes approximately 75% of the dose was recovered from the blood but after one hour only about 5% remained in the blood and at 24 hours the level had returned to normal. The levels in the lungs and kidneys were also highest immediately following injection and showed decreases with time similar to that in the blood. At 24 hours, however, the level in the lungs was still about ten times normal and in the kidneys almost twice normal. On the other hand levels of tocopherol in the liver increased steadily during the first hour after injection, reaching a maximum between one and two hours, and then decreasing steadily to levels 15 to 20 times normal at 24 hours.

In the tissues examined, the total recovery of tocopherol was about 90% at 5 minutes and this level gradually decreased with time to 10% at 24 hours.

---

* Prepared in pellet form by W. and R. Fletcher Ltd., Wellington.

* Both obtained from L. Light and Co. Ltd.

† Atlas Powder Company, Wilmington, Delaware.

TABLE 1. LEVELS OF TOCOPHEROL IN RAT TISSUE AT VARIOUS TIME INTERVALS AFTER THE INTRAVENOUS INJECTION OF α-TOCOPHERYL ACETATE

No. Rats	Time after Injection (h)	Blood (mg)	Liver (mg)	Lungs (μg)	Kidneys (μg)	Total (mg)
3	Control	0.13	0.11	3.5	9	0.2
4	1/12	14.5	1.8	1200	145	18
4	¼	10.0	2.9	670	105	14
4	½	4.2	6.0	335	110	11
4	¾	1.6	8.0	125	37	10
4	1	1.1	11.2	135	41	12
4	2	0.53	8.0	115	35	8.6
4	4	0.33	2.9	60	22	3.2
4	24	0.11	1.9	32	14	2.0

Injections of Tocopherol at Various Time Intervals Before and After Injections of Carotene

Table 2 shows the effect of preliminary or subsequent intravenous injections of 20 mg α-tocopheryl acetate as aqueous dispersions on the utilisation, as indicated by blood and liver vitamin A levels, of 400 μg

TABLE 2. THE EFFECT OF INTRAVENOUS INJECTIONS OF 20 mg α-TOCOPHERYL ACETATE ON THE LIVER AND BLOOD LEVELS OF VITAMIN A FORMED FROM 400 μg OF CAROTENE SIMILARLY ADMINISTERED

No. Rats	Treatment	Interval between Injections (h)	Blood Vitamin A Alcohol (μg/100 ml)	Liver Vitamin A		
				Alcohol (μg)	Ester (μg)	Total (μg)
2	None	—	8	0.3	0.6	0.9
6	Carotene alone	—	14	2.8	28	31
2	Carotene and tocopherol	Simultaneous	8	1.6*	4.0*	5.6
4	Carotene after tocopherol	1/12	9	2.2*	5.8*	8.0
2	ditto	¼	11	2.0	11	13
4	ditto	½	11	2.0	17	19
4	ditto	1	12	2.7	22	25
2	ditto	2	14	4.9	21	26
2	ditto	4	13	1.9	24	26
4	ditto	24	13	2.0	28	30
3	Tocopherol after carotene	1/12	14	2.3	9.0	11
2	ditto	¼	14	4.9	25	30
3	ditto	½	16	3.0	31	34
3	ditto	1	15	2.1	31	33
3	ditto	2	14	1.8	35	37
3	ditto	4	13	2.0	30	32

* Colour developed with antimony trichloride somewhat atypical.

β-carotene similarly administered. It is apparent that the efficiency with which the rat is capable of utilising intravenously administered carotene is depressed by a simultaneous or preliminary injection of tocopheryl acetate. The effect of the tocopherol decreases with time and conversion appeared to be normal when the injections were separated by 24 hours.

Tocopheryl acetate injected 5 minutes after the carotene depressed conversion to about one third normal, but when the injections were separated by 15 minutes or longer, no effect was apparent.

Total Vitamin A Formed at Various Time Intervals After Injections of Carotene

The total amount of vitamin A present in the rat carcass at various time intervals after the injection of carotene is shown in Table 3. Particularly at the shorter time intervals, difficulties were experienced in estimating vitamin A owing to the presence of an artifact which could not be separated from vitamin A alcohol and which produced, with antimony trichloride, an atypical non-fading colour. The vitamin A levels must therefore be regarded as approximate only but, whatever the absolute level, it is apparent that there is a steady increase in vitamin A in the whole carcass up to 24 hours after carotene injection.

TABLE 3. VITAMIN A AND CAROTENE IN WHOLE RAT CARCASSES AT VARIOUS TIME INTERVALS AFTER THE INTRAVENOUS INJECTION OF 400 μg β-CAROTENE
(One rat at each time interval)

Interval between Injection and Slaughter (h)	Carotene (μg)	Vitamin A (μg)
1/12	400	7.2
1/4	380	7.8
1/2	340	7.2
1	350	13
2	320	14
4	300	21
24	230	26
Control (no injection)	—	1.8

DISCUSSION

From the results presented in Table 2 it is clear that not only is the utilisation of intravenously administered aqueous dispersions of carotene markedly reduced by the simultaneous injection of tocopherol but that the effect of tocopherol persists, to a steadily decreasing extent, for some hours. Thus with the dosage levels used in the present investigation (400 μg β-carotene and 20 mg α-tocopheryl acetate), conversion, as indicated by liver stores of vitamin A 24 hours after the carotene injection, was about one-fifth normal when carotene and tocopherol were administered simultaneously, was about four-fifths normal when the carotene was injected one hour after the tocopherol, and was normal again when the interval between injections was increased to 24 hours.

In previous studies (Kon *et al.* 1955; McGillivray and Worker 1957) it has been shown that the level of vitamin A in the liver increases during the first 24 hours after injection. In the present investigation, although difficulties were experienced, owing to the presence of interfering artifacts, in measuring the total amount of vitamin A in the whole carcass, the results presented in Table 3 indicate a probable steady formation of the vitamin over a similar period. This observation would support the suggestion previously advanced by McGillivray and Worker (1957) that as long as reasonably high levels of carotene remain in the blood, vitamin A is formed at some extra-hepatic site and transported to the liver via the blood.

In view of this apparent formation of vitamin A over 24 hours, it was surprising to find that when tocopherol was injected after the carotene, liver storage of vitamin A was depressed only at the 5-minute interval and that conversion was normal when the injections were separated by 15 minutes or longer. The amount of vitamin A in the carcass half an hour after injection of carotene was only about one third of that present at 24 hours (Table 3) and it was expected that the injection at that time of tocopherol would depress the formation of further vitamin A. No such effect was apparent, and the most probable explanation for these observations is that there is an initial rapid breakdown of the carotene to an intermediate which is then more slowly converted to vitamin A. It is the formation of this intermediate that is probably inhibited by tocopherol, and the formation of vitamin A during the 24 hours following injection is dependent on the further breakdown of this intermediate rather than on a continued level of carotene in the blood. Indeed, as has been suggested for the pigment present in the liver (McGillivray and Worker 1957), carotene in the blood and in other tissues may, in the course of 15 minutes or less, have been converted to a physical state from which it is no longer available to the animal for conversion to vitamin A. Kon *et al.* (1955) have already shown that utilisation of intravenously administered carotene is intimately related to its physical state, and it is significant that, although the initial appearance of vitamin A in the blood and liver is extremely rapid (Kon *et al.* 1955), the vitamin A which finally appears in the liver represents only a very small proportion of the injected carotene (about 10% of a 400 μg dose). Although no information is available regarding the fate of intravenously administered Tween 40, it is possible that it could be metabolised sufficiently rapidly to explain a change in the physical state of the carotene.

Regarding the site of conversion to vitamin A of intravenously administered carotene, experiments in which various organs and tissues have been removed (McGillivray *et al.* 1956; Worker 1957b) have shown that conversion is not specifically sited in the liver, stomach, small intestine, large intestine, pancreas, kidney, adrenals, gonads, lungs, head, or neck. These results support the earlier conclusions of Bieri and Pollard (1954) and of Kon *et al.* (1955) "that perhaps many tissues possess the ability to convert carotene". However, experiments of this type tend to give qualitative rather than quantitative results owing to differences in blood volumes involved and the short time during which the animal can survive following acute surgery. Conversion may have been considerably reduced by the removal of some of these organs, and possibly in the intact animal one or more of these organs may in fact be a main site of conversion. However, on the basis of blood and liver levels of vitamin A at different time intervals after carotene injection, McGillivray and Worker (1957) considered that the liver is an unimportant site of conversion in the intact animal.

Further evidence regarding possible sites of carotene conversion can be obtained from the known effect of tocopherol and the rate at which it appears and disappears from tissues following intravenous injection. Since the reaction which tocopherol inhibits is a rapid one we are concerned only with the levels of tocopherol in the tissues during and immediately following carotene injection. The tissue levels of tocopherol shown in Table 1 therefore afford further evidence for rejecting the liver as a main site of conversion in the intact animal. The levels of tocopherol in the liver bear no relationship to the utilisation of carotene injected at increasing time intervals after the tocopherol, the level of tocopherol being low immediately after injection when the inhibition is greatest, and highest some two hours after injection by which time conversion is returning to normal. In the blood, kidneys, and lungs, however, levels of tocopherol are highest immediately following injection and show decreases with time similar to the decreasing effect of tocopherol on conversion. Although the correlation is closest in the case of blood tocopherol levels, the experiment hardly affords evidence for rejecting tissues such as lungs and kidneys in favour of blood as the site of conversion. It is significant, however, that, on the basis of other evidence, McGillivray and Worker (1957) have already drawn attention to blood as a site where conversion could at least be initiated, a random, rather than a specific, oxidation producing a fragment such as, for example, retinene which could then be converted in the blood or in other tissues to vitamin A.

Clearly further work is necessary to establish the site of conversion but all the evidence presented here is entirely compatible with the suggestion of the breakdown being initiated in the blood with the rapid formation of an intermediate such as retinene, or some other carotenal (Glover and Redfearn, 1954), which is then more slowly converted to vitamin A. All attempts in this laboratory to effect the conversion of carotene to vitamin A by incubation *in vitro* with blood have, however, failed nor have any likely intermediates been isolated from the blood of the intact animal after carotene injection or from the incubated material. The intermediate could however be a labile material which rapidly undergoes further oxidation unless removed from the blood by tissues where its conversion to vitamin A could occur.

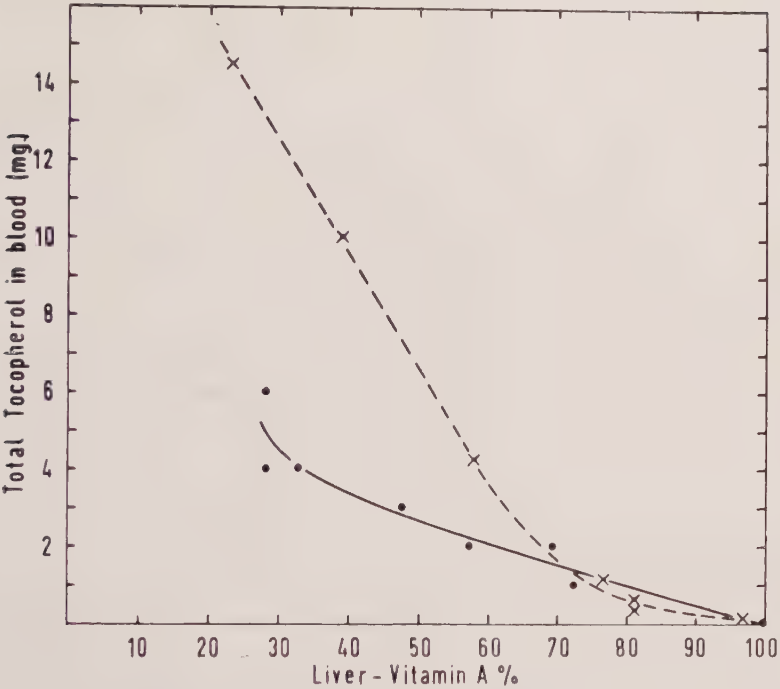


Fig. 1.—Relationship between blood levels of tocopherol at the time of carotene injection and the quantity of vitamin A appearing in the liver 24 hours later. **x - x** Tocopherol resulting from a preliminary injection of tocopheryl acetate. **. — .** Tocopherol levels resulting from the injection of varying amounts of tocopheryl acetate simultaneously with the carotene. (To allow for slight differences in the carotene dispersions, vitamin A is expressed as a percentage of that appearing in the liver 24 hours after the injection of carotene alone.)

The close negative correlation between blood tocopherol levels at the time of carotene injection and liver levels of vitamin A 24 hours later is shown in Fig. 1. Data for this figure are obtained from Tables 1 and 2. Also plotted on the same figure is the effect of the injection, simultaneously with the carotene, of comparable levels of tocopherol. These data are obtained from a previous publication (McGillivray and Worker, 1957). If it is only the level of tocopherol in the blood that influences conversion, it would be expected that a given level of tocopherol, whether injected with the carotene or resulting from a previous injection of tocopherol, would produce the same depression of conversion. It would appear however that tocopherol injected simultaneously, at least at the higher levels, is considerably more effective in reducing conversion. No explanation is offered for these differences unless it is again the physical state of the tocopherol which is involved.

Acknowledgements

We are grateful to the Department of Scientific and Industrial Research for a grant towards this investigation. The technical assistance of Miss Fay Frecklington with some of the experimental work is also acknowledged.

REFERENCES

BIERI, J. G.: 1955: *Proc. Soc. exp. Biol. Med. 88:* 482.

BIERI, J. G.; POLLARD, C. J. 1954: *Brit. J. Nutr. 8:* 32.

BIERI, J. G.; SANDMAN, R. P. 1951: *Fed. Proc. 10:* 376.

GLOVER, J.; REDFEARN, E. R. 1954: *Biochem. J. 58:* xv.

HEBERT, J. W.; MORGAN, A. F. 1953: *J. Nutr. 50:* 175.

KON, S. K.; MCGILLIVRAY, W. A.; THOMPSON, S. Y. 1955: *Brit. J. Nutr. 9:* 244.

MCGILLIVRAY, W. A.; THOMPSON, S. Y.; WORKER, N. A. 1956: *Brit. J. Nutr. 10:* 126.

MCGILLIVRAY, W. A.; WORKER, N. A. 1957: *Brit. J. Nutr. 11:* 47.

THOMPSON, S. Y.; GANGULY, J.; KON, S. K. 1949: *Brit. J. Nutr. 3:* 50.

WORKER, N. A. 1957a: *J. Sci. Food. Agric. 8:* 442.

——————1957b: *Brit. J. Nutr. 11:* 44.

NEW ZEALAND JOURNAL OF SCIENCE

Volume 1, No. 1, March 1958.

CONTENTS

The Estimation of Missing Values in Factorial Experiments. *G. M. Wright*

The Dichromate—Lignosulphonate Reaction and Its Potential Use in Chemical Grouting. *Allette Weston* and *R. A. Kennerley.*

The Steam Distillation of Volatile Fatty Acids. *R. O. Weenink.*

Bacteriological Survey of Auckland Harbours. Part 5. *G. M. Wallace, Lorraine E. Newman,* and *Jacqueline L. Jerrome.*

Optimum Arrays for Direction Finding. *N. F. Barber.*

Corrosion of Mild Steel in Some New Zealand Soils. *H. R. Penhale.*

Low-temperature Adsorption of Various Gases by Soils. *K. S. Birrell* and *R. Q. Packard.*

Nutritional Studies of *Phytophthora cactorum* (Leb. and Cohn) Schroet. Part 1. *T. Christie.*

Sulphur Isotopic Variations in Nature. Part 4. *J. R. Hulston* and *B. W. Shilton.*

Sulpher Isotopic Variations in Nature, Part 5. *T. A. Rafter, S. H. Wilson,* and *B. W. Shilton.*

Rapid Measurement of the Fibre Diameter of Romney Crossbred Wool by the Airflow Apparatus. *D. A. Ross.*

CONTENTS

PAGE

The Effect of Length of Pastures on Cobalt-deficiency Disease in Lambs. *E. D. Andrews, B. J. Stephenson, J. P. Anderson* and *W. C. Faithful* 125

Bloat in Cattle. VII. The Measurement of Foaming Properties of Surface-active Compounds. *J. L. Mangan* 140

Potassium Deficiency in Pastures. I. Potassium Content of Legumes and Grasses. *K. J. McNaught* 148

Fungal Leaf-spot Diseases of Cocksfoot (*Dactylis glomerata* L.) in the Manawatu. I. Leaf Streak Caused by *Scolecotrichum graminis* Fckl. *G. C. M. Latch* and *H. T. Wenham* 182

Surface Applicators for the Control of Lice and Keds on Sheep. *P. L. Thomas* 189

Root Development in Some Common New Zealand Pasture Plants. X. Effect of Pure Sowings of Some Grasses and Clovers on the Structure of a Tokomaru Silt Loam. *G. S. Robinson* and *W. A. Jacques* 199

The Control of Lice (*Damalinia ovis*) and Keds (*Melophagus ovinus*)—A Comparison of the Efficiency of Common Insecticides. *P. L. Thomas* 217

The Concentration of Radioactive Iodine by the Thyroid Glands of Normal Sheep under Various Conditions. *Evan Wright* 224

Superficial Scald: Some Methods of Control, Reported Overseas, Applied to New Zealand-grown Granny Smith Apples. *C. S. Padfield* 231

A Review of Work in New Zealand on the Clover Case-bearers (*Coleophora spissicornis* Haw. and *C. alcyonipennella?* Kollar (Coleophoridae, Lep.). *A. M. Stuart* 239

Studies in Hogget Rearing. I. General Characteristics of Hogget Ill-thrift. *E. A. Clarke* and *Daisy B. Filmer* 249

Eelworm (*Anguina agrostis* (Steinb.) Filipjer) in *Agrostis tenuis* Sibth. in New Zealand. *A. J. Healy* 265

Effects of Ingestion of Paraffins by Ruminants. IV. Effects of Paraffins and other Substances on the Utilisation of High Dosage Levels of Carotene. *W. A. McGillivray* and *N. A. Worker* 267

The Effect of Tocopherol on the Utilisation of Intravenously Administered Carotene. *W. A. McGillivray* and *N. A. Worker* 273

SUBSCRIPTIONS. Subscriptions will be accepted only on advance payment and only for a full calendar year. Price: 30s. per annum; single copy 5s.; post free (surface mail). Published in alternate months, starting February. Subscriptions should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 8018, Wellington, New Zealand. Cheques should be made payable to the Department of Scientific and Industrial Research, and if drawn on banks outside Wellington, must have exchange added. Subscribers are requested to give their name and full postal address.

This journal may also be ordered through The High Commissioner for New Zealand, 415 Strand, London W.C.2, or through any bookseller.

Printed by Coulls Somerville Wilkie Ltd.,
Wellington, New Zealand.

New Zealand Journal of Agricultural Research

VOLUME 1 No. 3
JUNE 1958

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind, however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. 4¼ × 7 in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is 4¼ × 7 in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation, etc., of references, see examples in this issue.

NEW ZEALAND JOURNAL
of
AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. A. Black.

VOL. 1. JUNE 1958 Number 3

SOME EFFECTS OF MCPB ON ESTABLISHING PASTURES

By D. B. EDMOND, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 2 December 1957*)

Summary

MCPB (sodium salt) was sprayed on four establishing, (i.e. newly sown) pastures of short-rotation ryegrass and clover in autumn 1956, and a study was made of some effects of several times and rates of application on plant numbers and growth form.

In only two situations were white-clover numbers reduced by 2 lb and 3 lb acid equivalent MCPB/acre, while red-clover numbers were reduced by one 3 lb application. These reductions may have been due in part to the action of MCPB products in the soil.

All treatments reduced weeds, seedling docks (*Rumex crispus* L. and *Rumex obtusifolius* L.) in the 2–4 leaf stage being killed by 1 lb MCPB, but mature docks required 3 lb MCPB.

Establishing pasture of short-rotation ryegrass and clover could be sprayed with 1 lb acid equivalent MCPB/acre, without significant effect on white or red clovers, provided the soil was covered by foliage.

INTRODUCTION

Some effects of spraying an established pasture with MCPA have been reported (Edmond 1955). Despite careful application of a light rate of the weedkiller, both white and red clovers (*Trifolium repens* L. and *T. pratense* L.) were damaged so that benefits from spraying were negated. With the development of the selectively activated weedkiller MCPB, i.e. 2-methyl-4-chlorophenoxybutyric acid (Wain 1955), it appeared that many weeds could be killed soon after emergence without concurrent damage to the clovers. As early removal of weeds from pasture is desirable, it appeared likely that MCPB would be particularly useful in the spraying of establishing pastures.

Accordingly, study was made of some effects of MCPB (sodium salt) on four establishing pastures, at different times and rates in May–July 1956, all the pastures being within a compass of 20 miles.

N.Z. J. agric. Res. 1: 281–289.

Experiment 1

In March 1956 seed was sown (Table 1) during dry weather in a reasonably fertile Manawatu sandy loam, which had been ploughed from a pasture infested with docks (mainly *Rumex obtusifolius* L.), of which a number had survived and re-established. Daily rain from 5 April promoted emergence commencing on 8 April.

TABLE 1. New Zealand Certified Seed Sown in Experimental Pastures (lb./acre)

	Experiment			
	1	2	3	4
Short-rotation ryegrass (*Lolium perenne* L. × *L. multiflorum* Lam.)	15	25	15	30
White clover	3	3	2	3
Red clover	0	4	3	4

Treatments using MCPB at the rate of 1 lb acid equivalent in 15 gal water/acre were:

1. Unsprayed.
2. Sprayed 2 weeks after emergence.
3. Sprayed 4 weeks after emergence.
4. Sprayed 1 day before 1st grazing (5 weeks after emergence).
5. Sprayed 1 week after 1st grazing (6 weeks after emergence).
6. Sprayed 4 weeks after emergence and also 1 week after 1st grazing.

Plots measured 5 ft × 60 ft, the treatments being randomised in seven replicates. Plant counts were used as criteria of treatment effect, four fixed quadrats 3 ft long being located in each plot. Docks and *Chenopodium album* L. were counted on a square yard, while others were counted on a square foot (3ft × 4 in), in all quadrats at each spraying and five weeks after the final treatment, dead plants being ignored.

When Treatment 2 was applied, the pasture was sparse, most clovers having one trifoliate leaf, but when Treatment 3 was applied two weeks later, the soil was almost covered by foliage. Recovery from an even cow grazing was rapid, but Treatments 5 and 6 were made with 30–40% bare soil. A second light grazing was made 4 weeks after Treatment 6 was applied.

Results

Initially, the white-clover population in Treatment 5 quadrats was greater than the mean, while those in Treatments 4 and 6 were

TABLE 2. EFFECT ON PLANT NUMBERS OF 1 lb ACID EQUIVALENT MCPB/ACRE APPLIED AT DIFFERENT TIMES (Mean counts per sq.yd.)

Time of Application (weeks after emergence)	White Clover				*Coronopus didymus*	Docks	*Sisymbrium officinale*
	4 weeks after emergence	5 weeks after emergence	6 weeks* after emergence	11 weeks after emergence	11 weeks after emergence	11 weeks after emergence	11 weeks after emergence
Unsprayed	122	126	92	38	61	61	14
2 weeks	113	79	63	29	7	9	0
4 weeks	113	115	70	27	14	22	Trace
5 weeks	104	106	83	29	9	25	Trace
6 weeks*	140	131	108	45	9	17	Trace
4 and 6 weeks	108	133	90	29	2	9	0
Sig. diff. at 5% level	16	25	20	11	27	11	5

* 1st grazing made 5 weeks after emergence.

smaller. When Treatment 4 was applied, it was clear that Treatment 2 had reduced white-clover number (Table 2). At the final count all sprayed white-clover populations had been reduced, but the unsprayed population had also decreased. *Coronopus didymus* (L.) Sm. declined from about 2,000 per sq. yd. to 10–250, and the difference between each MCPB treatment and unsprayed was highly significant. *Chenopodium album* L. reacted quickly and it appeared that all sprayed plants died. Docks and *Sisymbrium officinale* (L.) Scop. were affected by the spray, but later spraying was less effective. Generally speaking, Treatment 6, with its double application, was about as effective as Treatment 2, the first application.

A few days after spraying, distorted leaves appeared on sprayed white and red clover, but most of the plants persisted and developed normally. Where MCPB reached the soil, at spraying or by rainwash, short-rotation ryegrass plants were also affected. The leaves failed to open and there was no true "flag" while the habit of growth changed rapidly from a semi-erect tufted form to a relatively open, disordered, prostrate form. The herbage characteristically and clearly became a deep green colour, while vigour was reduced which, together with the reduced clover population noted above, led to reduced herbage yield at the first grazing (Table 3).

These MCPB effects disappeared after the 2nd or 3rd grazing.

Discussion

MCPB reduced weed numbers consistently, the application within 2 weeks of emergence being most effective, but docks with 4 or more leaves were incompletely controlled. The effect was similar to that of

TABLE 3. EFFECT OF 1 lb ACID EQUIV. MCPB/ACRE ON DRY-WEIGHT YIELD OF HERBAGE (ALL SPECIES) OF AN ESTABLISHING PASTURE
(g/sq.yd. at first grazing)

Treatment	Yield
1. Unsprayed	42.3
2. Sprayed 2 weeks after emergence	14.9
3. Sprayed 4 weeks after emergence	42.7
4. Sprayed 1 day before 1st grazing	42.9
5. Sprayed 1 week after 1st grazing	40.0
6. Sprayed as for 3 and 5	38.0
Sig. diff. at 1% level	11.4

MCPA, the palatability of MCPB-treated pasture being improved, but in this trial damage to clover was slight. Although the first application killed clover, it was soon possible to kill weeds without effect on clovers.

However, there was some distortion of leaves in sprayed plants of both red clover (Edmond 1956) and white clover, which did not appear to have any permanent effect on clover vigour, although it is possible that treated clover plants were temporarily less fit to enter pasture competition. The lower white-clover numbers at the final count (Table 2) might thus be explained in part, but grazing damage was important.

Where MCPB reached the soil, the vigour of all plants appeared to be reduced. Fortunately, short-rotation ryegrass appeared to regain normal vigour after the second or third grazing. Wain (1955) suggested that micro-organism enzyme activity in the soil might facilitate the β oxidation of MCPB, and Fletcher (1956) appeared to support this. Thus it is desirable to ensure that MCPB is applied to the foliage only.

Experiment 2

In the second week of April 1956, seed was sown (Table 1) in a reasonably fertile Manawatu silt loam which had been ploughed from a weedy turnip crop and in which a few docks survived. Emergence commenced about 15 April. Using MCPB at the rates of 0, 1, 2 and 3 lb acid equivalent in 15 gal water/acre, treatments were applied

(a) 3–4 weeks after emergence.
(b) 4–5 weeks after emergence.
(c) 5–6 weeks after emergence.

Plots measured 5 ft × 60 ft, treatments being randomised in a split-plot plan (rates in sub-plots) with four replicates. Five fixed quadrats were located in each plot, as in Exp. 1; plant counts were made in plots currently being sprayed and in all plots 8 weeks after the final treatment.

At first treatment the pasture was open, most clovers having one or two trifoliate leaves, while at the third treatment foliage covered the soil completely. The pasture was lightly grazed with cows a few days before the final count, the stock preferring the weed-free plots.

Results

TABLE 4. EFFECT ON PLANT NUMBERS OF MCPB APPLIED AT DIFFERENT TIMES AND RATES (Mean counts per sq.yd., 13–14 weeks after emergence)

Time of Application Weeks After Emergence	Docks		*Ranunculus* spp.	
	Unsprayed	Sprayed	Unsprayed	Sprayed
3 – 4	3.3	0.8	36.9	5.4
4 – 5	3.3	1.4	36.0	7.2
5 – 6	3.0	0.8	38.7	3.6
Rate of Application	Overall Means		Overall Means	
Unsprayed	3.2		36.9	
1 lb acid eq./acre	1.2		8.1	
2 lb acid eq./acre	0.9		3.6	
3 lb acid eq./acre	1.0		4.5	
Sig. diff. at 5% level	0.4		7.2	

In general, mature docks withstood the treatment, but rain after the 1st and 3rd spraying increased the MCPB effect (Table 4). *Ranunculus* spp. reacted in a similar way, as did white clover, but the effects on the latter were not significant. Subsequently, distortion of clover leaves was most noticeable at the 3 lb rate, there being early traces of scorch on red-clover leaves. The MCPB effect through the soil was less pronounced than in Exp. 1. Both *Chenopodium album* L. and *C. murale L.* were killed by MCPB.

Discussion

There did not appear to be any real advantage in applying 2 or 3 lb acid equivalent MCPB/acre, although after 12 months it was seen that the 3 lb rate had been most effective on docks. It could be argued that rain water leached MCPB and its breakdown products from the surface soil, thus reducing the effect through the soil, but the superior effect of rain-washed treatments on docks and *Ranunculus* spp. did not support this. Soil and air temperatures probably were similar to those operating in Exp. 1.

There was no significant difference in number of white-clover plants, but treated plants, particularly those in the first spraying, appeared to be reduced in vigour. This supports the suggestion that foliage should cover the soil at spraying.

Experiment 3

In late March 1956, seed was sown (Table 1) in a reasonably fertile Kairanga silt loam which had been cropped with potatoes and was free from mature weeds. Emergence commenced about 10 April 1956.

Using MCPB in 15 gal water/acre, treatments were applied

(a) 3–4 weeks after emergence, with 0, 0.4, 0.8 and 1.2 lb acid eq./acre.

(b) 4–5 weeks after emergence, with 0, 1, 2, and 3 lb acid eq./acre.

(c) 5–6 weeks after emergence, with 0, 1, 2 and 3 lb acid eq./acre.

Plots measured 5 ft × 100 ft, treatments being randomised as for Exp. 2, the final count being made 7 weeks after the final treatment. Slow early establishment permitted much of the first application to reach the soil, but subsequent vigorous growth caused the soil to be covered by foliage at the final treatment. There was some rain wash after the final treatment. Mobbed sheep grazed the pasture evenly ten days after the final treatment and again before the final count.

Results

Light early spraying reduced weed species and, so far as was measured, the white-clover population was unaffected. Later sprayings, particularly at heavy rates, were more effective on weeds but reduced the white-clover population (Table 5). All the seedling docks were affected

TABLE 5. EFFECT ON PLANT NUMBERS OF MCPB APPLIED AT DIFFERENT TIMES AND RATES (Mean counts per sq.yd., 12–13 weeks after emergence)

Rate of Application	Application 3–4 Weeks after Emergence		
	Docks	*Ranunculus* spp.	Thistles
Unsprayed	1.6 ± 0.7	34	8 ± 3
0.4 lb acid eq./acre	0.6 ± 0.3	16	3 ± 3
0.8 lb acid eq./acre	0	3	3 ± 3
1.2 lb acid eq/acre	0.1 ± 0.3	0	4 ± 3
Sig. diff. at 5% level	N.S	9	N.S

	Mean of Applications 4–5 and 5–6 Weeks after Emergence			
	White Clover	Docks	*Ranunculus* spp.	Thistles
Unsprayed	52	1.4	55	12
1 lb acid eq./acre	34	Trace	1	0.2
2 lb acid eq./acre	24	0	0	0.4
3 lb acid eq./acre	19	0	0	0
Sig. diff. at 5% level	8			

by MCPB, as were seedlings of *Cirsium arvense* (L.) Scop. Red clover was reduced in the 2nd and 3rd sprayings but there was no statistical significance. It appeared that 0.8 lb acid equiv./acre applied early was adequate for weed control.

The various effects of MCPB on clover and ryegrass plants noted in Exp. 1, were observed in this trial also.

Discussion

In general, the only extra effect of heavy rates of MCPB was reduction of white clover. Once more it appeared that successful spraying required application of MCPB to the foliage only. This was emphasised by the death of affected white-clover plants when competition became intense before the first grazing. It is probable that weeds treated with light applications of MCPB may react to intense competition in the same way.

Experiment 4

In late March 1956, seed was sown (Table 1) in a fertile Halcombe silt loam soil, which had been cropped with chou moellier and in which some docks persisted. Emergence commenced about 5 April 1956.

Treatments and procedure were the same as for Exp. 2 except that plots measured 5 ft × 80 ft, and the final count was made 3 weeks after the final treatment.

The pasture established rapidly, 60% of the soil being covered by foliage at the first treatment, while cover was about 100% at the second treatment. Rain wash occurred after the 2nd application and to a lesser extent after the 3rd. Dairy cows grazed the pasture evenly two weeks after the final treatment.

Results

The heavy rates of MCPB reduced the white-clover population at the 2nd and 3rd sprayings (Table 6) while docks were affected most by the 2nd spraying and *Ranunculus* spp. least by the third spraying. Ryegrass and clovers were affected as in Exp. 1, after the 1st and 2nd sprayings, particularly the latter because of rain wash.

Discussion

Despite the early date of the final count, most of the MCPB effect had developed and the pattern was as for the other experiments with an exception where low-growing *Ranunculus* spp. were affected less. In this instance the dense foliage cover probably restricted the coverage of the plants thus reducing the effective rate of application. It is possible that the second treatment might have given the best result, but, as it was, rain wash increased deaths of clover and docks. In general, the 1 lb rate was effective on the weeds, but mature docks required the 3 lb rate. The heavy rates possibly reduced the ability of sprayed clover plants to withstand intense plant competition, which operated from the 2nd spraying on.

TABLE 6. Effect on Plant Numbers of MCPB Applied at Different Times and Rates (Mean counts per sq.yd., 8–9 weeks after emergence)

Rate of Application	Red Clover	White Clover		
	Overall Means	3–4 weeks after emergence	4–5 weeks after emerg	5–6 gence
Unsprayed	12	17	21	20
1 lb acid eq./acre	14	22	22	22
2 lb acid eq./acre	14	23	18	19
3 lb acid eq./acre	11	19	13	15
Sig. diff. at 5% level	1.4	4.5	4.5	4.5

Rate of Application	Docks			*Ranunculus* spp.		
	3–4 weeks after emergence	4–5 weeks after emergence	5–6 weeks after emergence	3–4 weeks after emergence	4–5 weeks after emergence	5–6 weeks after emergence
Unsprayed	1.4	1.2	1.5	37	29	24
1 lb acid eq./acre	0.6	0.1	0.6	0.7	2.3	12.4
2 lb acid eq./acre	0.5	0.1	0.5	3.1	0	10.8
3 lb acid eq./acre	0.3	0.1	0.1	0	0.9	11.5
Sig. diff. at 5% level	0.3	0.3	0.3		1 v 3 2 v 3	2.5 2.0

General Discussion and Conclusions

A number of the MCPB treatments reported here have favoured vigorous growth of short-rotation ryegrass and red and white clovers. A rate of 1 lb acid equivalent/acre was effective on all weeds present except *Rumex* spp. with 4 or more leaves, and many of these were controlled by 3 lb acid eq./acre. Light applications a few days after emergence were about as effective as the 1 lb rate applied a few weeks later, but this course was dangerous for much MCPB reached the soil and MCPB action through the soil was non-selective. This danger might have been increased if repeat applications had been required.

Using normal equipment, it was possible to apply MCPB to the foliage only, as soon as foliage covered the soil—a matter of 4 or 5 weeks after emergence in some pastures. Where this point was ignored, clover numbers and vigour were reduced, but there was increased effect on the weeds. Successful applications were possible during fine weather in the autumn, but sodium salt of MCPB could be washed through to the soil, and formulations that adhere to the foliage might be preferred.

It was observed that clover plants, particularly those newly emerged, with scorched and distorted leaves, were checked by pasture competition. [illegible] rmal leaves were numerous the check was slight.

[illegible] eeds in the autumn appeared within 1–3 days and in gene[illegible] lly developed within 7–14 days. Thus, in rapidly establishing [illegible] es, sprayed weeds could offer competition, but this did not appear to be serious where the treatments were applied prudently. Delayed applications permitted weed competition to operate and weed tolerance to MCPB to increase, while it became more difficult to spray all leaves without forcing MCPB through to the soil.

Using dock tolerance as a criterion, MCPB appeared to be more effective than could be expected for equivalent MCPA.

It is concluded that MCPB (sodium salt) at the rate of 1 lb acid equivalent/acre effectively kills weeds in establishing pasture, without appreciable damage to white and red clovers, provided foliage covers the soil. Applications made 5–7 weeks after emergence were successful in these trials. Difficult weeds such as mature docks may also be killed by increasing the application, but some clover damage may result.

ACKNOWLEDGEMENTS

Messrs. F. T. Baxter, D. Burke, M. Williamson, and the Dairy Research Institute (N.Z.) provided the pastures; Misses D. P. Owen, D. Murphy, and M. Cummins counted the plants; and Mr. A. C. Glenday, Applied Mathematics Laboratory, assisted in statistical matters.

REFERENCES

EDMOND, D. B. 1955: The Effect on an Establishing Pasture of Spraying with Sodium 2-Methyl-4-Chlorophenoxyacetate. *N.Z. J. Sci. Tech. A36:* 548-56.

——————1956: The Effect of MCPB on Young Red Clover and Weeds. *Ibid. A38:* 397-402.

FLETCHER, W. W. 1956: Effect of Hormone Herbicides on the Growth of *Rhizobium trifolii. Nature 177:* 1244-5.

WAIN, R. L. 1955: A New Approach to Selective Weed Control. *Ann. appl. Biol. 42:* 151-7.

APPENDIX

Where MCPB appeared to be applied to the foliage only, plants newly observed to be activators were:—

Amaranthus retroflexus L.
Chenopodium murale L.
Cirsium arvense (L). Scop.
Cirsium lanceolatum (L.) Hill
Polygonum aviculare L.
Ranunculus spp.
Rumex crispus L.
Silybum marianum (L.) Gaertn.
Sonchus oleraceus L.

LABORATORY METHODS FOR MEASURING THE TOXICITY OF PESTICIDES TO HONEY BEES

By T. PALMER-JONES, Animal Research Station, Department of Agriculture, Wallaceville

(*Received for publication, 13 November 1957*)

Summary

A description is given of laboratory tests designed to study the effect upon honey bees of pesticides acting as stomach and dry contact poisons. Methods are also given for measuring direct spray and fumigant action.

The ascending order of toxicity to bees of field applications of the pesticides tested is considered to be toxaphene, strobane, thiodan, Primin, and diazinon.

Field applications of toxaphene and strobane would be expected to cause little mortality if made after bee activity has ceased; but applications of thiodan, Primin, and diazinon would be expected to cause heavy mortality.

It must be emphasised that these predictions are based on laboratory tests, and field trials must be carried out before toxaphene and strobane can be considered safe to bees when applied under New Zealand conditions.

Compound 1080 is highly toxic to bees as a stomach poison.

INTRODUCTION

Many laboratory techniques have been devised for measuring the toxicity of pesticides to honey bees. The variety of methods used and the different interpretations put on the results obtained have often been the cause of conflicting claims regarding the potential danger to bees of applications of pesticides. Eckert (1949) and Hafliger (1949) have both discussed this question, which is also referred to by Jones and Connell (1954).

Usually such tests are made on worker honey bees withdrawn from a hive and so do not measure the indirect effects of a pesticide upon a colony; it may be stored in pollen or honey and cause mortality months later; bees may bring sufficient of it into the hive on their bodies to have a fumigant or contact effect.

When pesticides are applied in the field, their effect on bees depends on weather conditions before and after application, on repellency, on method of application, on systemic effect, and on other factors which cannot be duplicated in the laboratory. The methods used by bees in collecting nectar and pollen from different types of flowers vary and so the type of plant to which the pesticide is applied may have some bearing on its effect.

Field trials of the type carried out by Palmer-Jones *et al.* (1954, 1957) on DDT and Metasystox must show no mortality before a new pesticide can be considered safe to bees. But because they are time-

N.Z. J. agric. Res. 1: 290–300.

consuming and costly, it is usually not practicable to apply them at short notice to all pesticides as these come on the market. It is not advisable in New Zealand to rely on the results of overseas trials because our climatic conditions, the type of crop to which the pesticide is applied, and the method of application, are usually different. Hence, the preliminary screening of new pesticides in the laboratory can be of particular value to us.

It is evident that caution must be used when considering, on the basis of laboratory tests, how field applications of a pesticide will affect bees. However, such tests provide a quick and cheap means of obtaining information on newly developed pesticides.

Those with least toxicity to bees can then be tested in field trials, particularly against aphids infesting brassicas and the clover case-bearer moth, *Coleophora spissicornis* and *C. alcyonipennella?*, and their effect on these pests and honey bees studied.

The tests now described provide a means of rapidly assessing the probable field toxicity to bees of pesticides.

INFORMATION QUOTED FROM LITERATURE ON PESTICIDES TESTED

Except in the case of toxaphene, little information was available regarding the toxicity of these to bees.

Thiodan

Thiodan is a sulphur-containing heterocyclic hydrocarbon compound (1,2,3,4, 7,7-hexachlor-bicyclo- (2,2,1) -hepten-5,6-bisoxymethylene sulphite). Its properties are still being investigated but it is claimed to have proved consistently good for the control of aphids and other insects; mammalian toxicity appears to be no greater than for DDT. It is applied at up to 2 lb per acre (Niagara Chemical Division 1957).

Finkenbrink (1956) claims that thiodan is non-injurious to bees; concentrations proposed by him for the control of forty-six pests vary from 0.02% to 0.1%.

Strobane

This is the chlorination product of a mixture of terpene hydrocarbons and has a chlorine content of approximately 66%. Trials which are not yet complete are claimed to show that it is effective against many species of household and agricultural insect, (Kent 1953), while being considered one of the safest to human beings of the synthetic organic insecticides (B. F. Goodrich Chemical Co. 1954). The suggested application on insect-infested plants is up to 2 lb of active material per acre.

Primin

Isolan is the proposed common name for the active ingredient, i.e. "Primin" is the proprietary brand name given by J. R. Geigy to a product containing 6% isolan. Isolan is a heterocyclic carbamate with systemic properties. It has the formula dimethyl 5-(1-isopropyl-3-methylpyrazolyl) carbamate.

Aphids are controlled by a concentration of 0.006%. The toxicity of isolan to warm-blooded animals is comparable to that of parathion and the same precautionary measures should be taken when applying both products.

Diazinon

Diazinon is 0,0-diethyl 0-(2-isopropyl-6-methyl-4-pyrimidinyl) thiophosphate. It is considered from many tests that diazinon can be used without any danger to operators (Geigy Insecticides 1954). Diazinon is sometimes prepared in emulsions with a concentration as high as 20% but the usual strength used against insects is 0.1% (J. R. Geigy 1954), applied at the rate of 1 oz of active material per acre. It was found that when 0.1% diazinon was sprayed on open flowers these were toxic to honey bees for up to 24 hours following application. So it is recommended that diazinon should not be sprayed on open blossom (Maurizio and Schenker 1953). Atkins and Anderson (1954) concluded, as a result of laboratory tests of the relative toxicity of pesticides, that diazinon was highly toxic to bees.

Toxaphene

Toxaphene has the formula $C_{10}H_{10}Cl_8$ and is a chlorinated camphene. It has been reported by many workers (e.g. Knowlton *et al.* 1950; Weaver 1953) that it can be applied to flowering plants with relative safety to bees. It would be applied at up to 2 lb of active material per acre.

Sodium Fluoroacetate (Compound 1080)

This potent rat- and mouse-killing agent is used in New Zealand for the destruction of rabbits and opossums. It was first applied in jam baits which are attractive to honey bees. When it was found at Wallaceville that the baits were highly toxic to bees, the lethal dose of 1080 as a stomach poison to bees was determined. Because of the co-operation of those applying the baits these are not now laid near apiaries at a time when bees are actively foraging and no losses have occurred. The use of jam baits is declining in favour of pellets and carrots impregnated with 1080, which do not attract bees.

EXPERIMENTAL

General

Adult worker honey bees (*Apis mellifera*) were used in the tests. They were withdrawn from strong hives of Italian hybrids by placing small cages over openings in the entrance blocks. The hives were then tapped if necessary and the issuing bees trapped in the cages by closing their entrances with slides.

The bees were confined while under observation in cages 3 × 3 × 1½ in. deep with nylon-mesh sides; 33⅓ W/V sucrose in feeders was always available to them. The cages were kept in 30°c incubators at a relative humidity of approximately 25%. Mortality is negligible in control bees kept under such conditions for three days. CO_2 was used as an anaesthetic in handling the bees.

The pesticides used in the tests were all commercial preparations (Table 1) and the presence of solvents, a sticker, and emulsifiers in dilutions might have some affect on bees. However, these substances would have a similar effect in field application.

TABLE 1. FORMULATION OF PESTICIDES

Pesticide	Formulation of Undiluted Pesticide
Thiodan or HOE 2671	20% W/V. Emulsifier. Xylene.
Strobane	40% W/V. Emulsifier. Xylene.
Primin	6% isolan W/V. Emulsifier.
Diazinon	16% W/V. Emulsifier. Xylene.
Toxaphene	50% W/V. Sticker and emulsifier. Xylene.
Sodium fluoroacetate	Technical grade.

Pesticides may affect honey bees in a variety of ways. They may act as stomach poisons, contact poisons (dry or wet), and fumigants. Also, bees may be sprayed or powdered with pesticides during field applications.

The following tests were designed to study the effect of pesticides as:

Stomach Poisons

Bees were withdrawn from a hive, starved for one hour, and then placed singly in small vials with a feeding tube at one end (Fig. 1). This tube held 0.02 ml of a sucrose solution containing a known strength of pesticide. The feeder was charged from an Agla micrometer syringe. Bees that regurgitated or did not consume all their dose were discarded. After dosing, the bees, usually 16-20 in number, were transferred to an

TABLE 2. TOXICITY OF PESTICIDES AS STOMACH POISONS

Pesticide	LD 50% in μg
Thiodan	4.4
Strobane	25
Primin	0.38
Diazinon	0.24
Toxaphene	20
Sodium fluoroacetate	0.8
Metasystox*	0.5

* See Palmer-Jones *et al.* 1957.

observation cage with a feeder and mortality noted after 24 hours. The LD 50% was obtained by dosing bees with a series of concentrations (Table 2). The method closely follows that described by Glynne Jones and Connell (1954).

Contact Poisons

This test was designed to find the effect on bees of remaining in contact with a dry film of a pesticide such as might occur on a crop.

An Aerograph Type MP spray gun was used to spray the pesticide evenly over one side of a sheet of tin foil 600 sq cm in area. A dye was added to the pesticide solution to aid in spraying it evenly. The foil was weighed before the spray was applied and after it had been dried in an oven at 33°c. Tin foil of the type used in bacteriology was found

TABLE 3. CONTACT EFFECT OF PESTICIDES

Pesticide	Grammes of Pesticide on 600 sq cm of Metal Foil	Equivalent Field Application, oz/acre	Mortality of Bees (%)
Thiodan	.0102	24	100
	.0062	15	68
	.0060	14	94
	.0042	10	20
	.0028	7	22
	.0016	4	14
	.0016	4	10
	.0014	3	6
	.0008	2	2
Strobane	.0246	59	76
	.0218	52	45
	.0176	42	74
	.0128	30	24
	.0118	28	2
	.0066	16	2
	.0034	8	0
	.0016	4	0
Primin	.0054	13	100*
	.0016	4	100*
	.0011	3	100*
	.0010	2	100*
	.0009	2	72
	.0008	2	94
	.0007	2	32
	.0006	1	0
Diazinon	.0454	108	100*
	.0266	63	100*
	.0034	8	100*
	.0028	7	100*
	.0010	2	94
	.0006	1	10
	.0004	1	2
	.0002	½	2
Toxaphene	.1006	239	100
	.0984	234	100
	.0690	164	100
	.0404	93	34†
	.0390	93	42†
	.0314	75	24†
	.0205	49	22†
	.0146	35	4
	.0126	30	4
	.0124	30	0
	.0120	29	0
	.0114	27	0
	.0094	22	0
	.0090	21	0
	.0034	8	0

* Bees all died during exposure.

† Surviving bees severely affected.

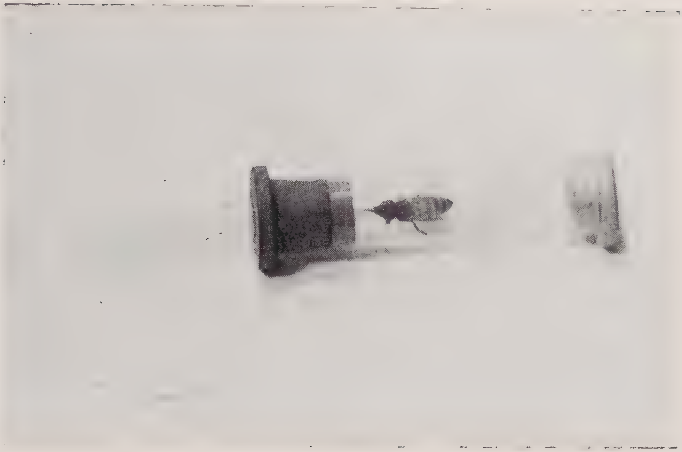


Fig. 1.—Bee consuming measured volume of pesticide.

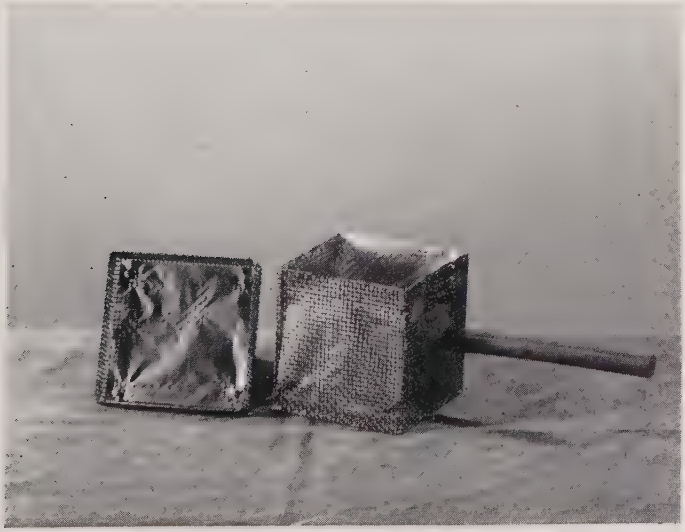


Fig. 2.—Foil-lined cage for dry-contact test.

much superior to grease paper for this purpose as it does not absorb moisture from the air. The foil was used to line completely the inside of a square wire cage with press-on lid (Fig. 2). Fifty bees that had first been fed for approximately one hour were then caged and kept in contact with the dried pesticide for one hour without syrup. During this period air was sucked into and out of the cage by a tube attached to a water pump. This prevented vapour from the pesticide building up and acting as a fumigant. After treatment, the bees were removed, kept for 24 hours in a cage supplied with syrup, and mortality noted. The weights of toxaphene shown in Table 3 are slightly in excess of those actually deposited because the solution of this contained a sticker.

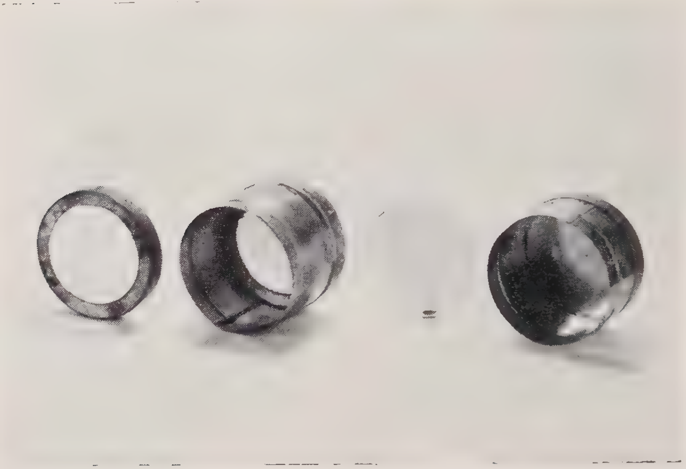


Fig. 3.—Device for studying fumigant effect.

Fumigants

Many pesticides, even if applied in the evening, after bees have ceased visiting a crop, will have some fumigant effect the next day.

Fumigant effect was studied by using circular holders, 4 in. across and 2.5 in. deep, with tops and bottoms made of wire gauze. Each holder was made with a flange that could be slid over the top of a tin which held a petri plate in which fitted a 3.5 in. filter paper (Fig. 3). These were soaked with 1 ml of the particular dilution of the pesticide under test and the holder, containing 50 bees, fitted above. The whole was then placed in a 30°c incubator and the bees exposed to a vapour for one hour. Afterwards they were transferred to cages with feeders and kept under observation for 24 hours, and mortality was noted.

Each dilution of a pesticide was tested upon four holders of bees. Bees were fed before exposure to the vapour, but no syrup was given during exposure. It was found that bees that had fed lightly before exposure to the vapours were less severely affected than those that had fed heavily. So, in each batch of four, two lots of bees were fed sugar syrup for 25 minutes and two for a minimum of 1.5 hours, representing light and heavy feeding. It will be seen from Table 4 that the former group which is always placed first in the columns usually shows much less mortality.

Sprays

The pesticides under test would generally be applied in the evening when bees had ceased flying. However, through carelessness or exigency, they might be put down earlier in the day and cause mortality in foraging bees. So their spray effect on bees was studied by a method developed previously by Palmer-Jones (1950).

TABLE 4. TOXICITY OF PESTICIDES AS FUMIGANTS
(% mortality for each cage in group of four treated)

	$0.1\%\frac{W}{V}$	$0.5\%\frac{W}{V}$	$1.0\%\frac{W}{V}$	$1.5\%\frac{W}{V}$	$2.0\%\frac{W}{V}$	$4\%\frac{W}{V}$	$5\%\frac{W}{V}$
Thiodan		0, 0, 0, 0					0, 0, 0, 6
Strobane		0, 0, 0, 0				0, 2, 2, 4	8, 14. 6, 6.
Primin	2, 2, 10, 0	4, 0, 60, 68	100, 86, 100, 100	48, 56, 80, 92	88, 96, 100, 100		
Diazinon	0, 2, 100, 100	100, 32, 100, 100	60, 70, 100, 100				
Toxaphene		0, 2, 0, 0	0, 0, 0, 2	0, 0, 0, 0	2, 2, 0, 0		2, 6, 0, 6

Batches of 50 bees, without preliminary feeding, were anaesthetised with CO_2 and spread over a 5 in. filter paper in a petri plate. While unconscious they were sprayed with 1 ml of the particular dilution of pesticide under test. The head of a No. 15 De Vilbiss atomiser was adapted for this purpose to fit into the top of a graduated cylinder from which a known volume of pesticide could be delivered as spray (Fig. 4).

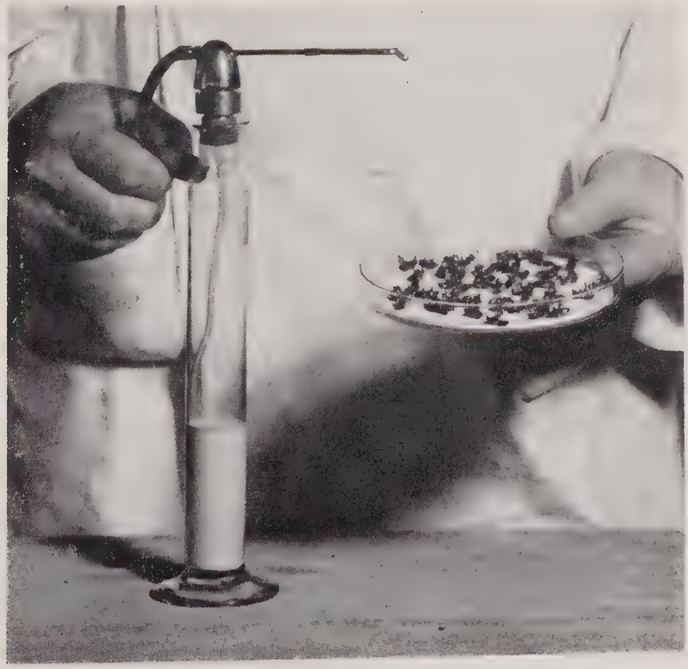


Fig. 4.—Device for studying spray effect.

Four batches, each of 50 bees, were sprayed with the dilutions of the pesticide under test. Sufficient spray was delivered to wet the bees thoroughly.

After spraying, the bees were caged with feeders, and mortality was noted after 24 hours (Table 5).

TABLE 5. TOXICITY OF PESTICIDES AS SPRAYS
(% mortality for each cage in group of four treated)

	0.1% $\frac{W}{V}$	0.5% $\frac{W}{V}$	1.0% $\frac{W}{V}$	1.5% $\frac{W}{V}$	2.0% $\frac{W}{V}$
Thiodan	0, 2, 0, 0,	6, 54, 22, 20	20, 36, 80, 96	100, 100, 100, 100*	
Strobane	0, 0 0, 0	4, 0, 0, 40	90, 60, 70, 80	100, 98, 100, 94	100, 100, 100, 100
Primin	100, 100, 100, 100†				
Diazinon	100, 100 100, 100†				
Toxaphene	0, 2, 0, 0	6, 8, 12, 20	96, 100, 100, 100	100, 100, 100, 100	

* Bees dead and dying within 2 hours of treatment with 1.5%.

† Bees dead and dying within half hour.

DISCUSSION

TABLE 6. SUMMARY

Pesticide	LD 50% in μg	Minimum Field Application Liable to Cause Mortality by Dry Contact (oz/acre)	Maximum Field Application Likely in Practice (oz/acre)	Minimum Concentration W/V Liable to Cause Mortality by Fumigant Action (%)	Minimum Concentration W/V of Spray Liable to Cause Mortality (%)
Strobane	25	30	32	5	1
Toxaphene	20	49	48	5+	0.5
Thiodan	4.4	4	32	5+	0.5
Primin	0.38	2	1	0.5	<0.1
Diazinon	0.24	2	1	0.1	<0.1
Sodium fluoroacetate	0.8				
Metasystox	0.5				

If pesticides are likely to cause bee mortality, they will generally be applied to flowering crops in the evening or early morning, when bees are not flying, so as to avoid losses through direct contact and reduce fumigant action. The contact effect does not usually persist for long under field conditions. But pesticides may also affect bees as stomach poisons through becoming dissolved in nectar or water, or contaminating pollen; hence, when evaluating the results summarised in Table 6, more attention should be paid to the effect of the pesticides as stomach and contact poisons than to their effect as fumigants and sprays.

Toxaphene furnishes a useful control as its effect on bees has been fully investigated in laboratory and field. It would be predicted from Table 6 that toxaphene is likely to prove safe to bees in field applications if applied after bee activity had ceased. This has been shown to be so in practice. The figure of 20 μg for the LD 50% of toxaphene agrees well with Eckert's (1949) figure of 22.

Field applications of strobane made after bee activity had ceased would also be expected to cause little mortality; but applications of thiodan, Primin, and diazinon would be expected to cause heavy mortality.

It must be emphasised that these predictions are based on laboratory tests, and field trials must be carried out before toxaphene and strobane can be considered safe to bees when applied under New Zealand conditions.

It is unlikely that many pesticides will be applied as dusts in New Zealand. In the case of aerial application of sprays the carrying capacity of the lighter planes used varies from 60 to 70 gal and from 7 to 10 gal per acre is the most economic volume of spray to apply. So, as the actual weights of pesticides applied per acre range from 1 to 3 lb, the solutions sprayed generally vary from about 1% to 3% W/V. Much the same strength would be used in ground applications. It is apparent from Table 6 that the spray of all the pesticides tested would cause bee mortality. Fumigant action would appear unlikely to cause mortality except in the case of Primin and diazinon.

Many bees that live for 24 hours after exposure to the dry contact effect of the pesticide are nevertheless weakened and would not survive under field conditions, so equivalent field applications that give a mortality of more than 10% (Table 3) are used to compile the second column of Table 6.

There was no evidence that lethal doses of the pesticides dissolved in sugar syrup exerted repellency. It was found that fumigant effect is much more pronounced amongst bees that have fed heavily than amongst those that have fed lightly.

The ascending order of toxicity to bees of field applications of the pesticides tested is considered to be toxaphene, strobane, thiodan, Primin, and diazinon.

The tests are easily carried out and provide a useful practical means of rapidly screening newly developed pesticides.

ACKNOWLEDGEMENTS

I am indebted to Mr. R. Odinot, Therapeutant Specialist, Horticulture Division, Wellington, for information covering the pesticides tested; Mr. A. L. Bryant, Photographer, Animal Research Station, Wallaceville, took the photographs used as illustrations: Messrs. Ivon Watkins Ltd., New Plymouth, and Messrs. Ivory Spray Chemicals Ltd., Nelson, were most helpful in providing samples of the pesticides tested and literature concerning them.

REFERENCES

ANON, 1955: *Rev. franc. Apic. 3(113):* 1322.
ATKINS, E. L. Jr.; ANDERSON, L. D. 1945: *J. econ. Ent. 47(6):* 969.
ECKERT, J. E. 1949: *Ibid. 42(2):* 261.
FINKENBRINK, W. 1956: *NachrBl. dtsch. PflschDienst., Braunschweig, 8:* 183.
GEIGY INSECTICIDES, 1954: *Toxicology of Diazinon.*
GEIGY, J. R., BASEL, 1954: *Diazinon in Plant Protection.*
B. F. GOODRICH CHEMICAL CO., OHIO. 1954: *Bulletin AD-1.*
HAFLIGER, E. 1949: *J. econ. Ent. 42(3):* 523.
JONES, GLYNNE G. D.; CONNELL, JOAN U. 1954: *Ann. appl. Biol. 41:* 271.
KENT, D. L. 1953: Paper read at Chemical Specialities Manufacturers' Assn., Chicago, Ill., 18 May.
KNOWLTON, G. F.; NYE, W. P.; LIEBERMAN, F. V.; TODD, F. E.; BOHART, G. E. 1950: *Fm Home Sci. 11(1):* 17.
MAURIZIO, A.; SCHENKER, P. 1953: *Mitt. Schweiz. ent. Ges. 26(4):* 305.
NIAGARA CHEMICAL DIVISION, FOOD MACHINERY AND CHEMICAL CORPORATION, MIDDLEPORT, NEW YORK. 1957: *Technical Information Niagara Thiodan.*
PALMER-JONES, T. 1950: *N.Z. J. Agric. 80(2):* 129.
PALMER-JONES, T.; BARTRUM, F. A.; FORSTER, I. W.; HARRISON, D. L. 1954: *N.Z. J. Sci. Tech. A36:* 177.
PALMER-JONES, T.; FORSTER, I. W.; GRIFFIN, L. A. M. 1957: *Ibid. A38:* 752.
WEAVER, NEVIN. 1953: *Texas agric. Exp. Sta. Progr. Rep. 1554.*

THE RELATIONSHIP OF *Olpidium brassicae* (Wor.) Dang. TO THE BIG-VEIN DISEASE OF LETTUCE

By P. R. Fry, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(*Received for publication, 16 January 1958*)

Summary

Inoculation of lettuce with tobacco-necrosis virus isolated from big-vein plants did not cause big-vein symptoms.

Olpidium brassicae was always found in roots of lettuce grown in big-vein-infected soil but not in two gardens where big vein did not occur.

Olpidium resting spores were sedimented from big-vein root sap by low-speed centrifuging. Inoculation of lettuce with resuspended sediment produced big-vein symptoms while inoculation with the supernatant liquid did not.

Veronica tournefortii Gmel., *Stellaria media* (L) Vill., and *Spergula arvensis* L. were found to be hosts of *O. brassicae.* Inoculation of lettuce with root sap of these species gave rise to big-vein symptoms.

Soil treatment with captan, phygon, copper oxychloride, and thiram, all reduced incidence of big vein.

These results suggest that *O. brassicae* is the cause of big-vein disease of lettuce.

The big-vein disease of lettuce was first recorded by Jagger and Chandler (1934) who found it was soil borne and that it could be controlled by partial sterilisation of soil with steam or formaldehyde. They suggested that the agent of big vein might be a virus similar to the soil-borne wheat-mosaic virus. Doolittle and Thompson (1945) reported that infection could be transmitted by mechanical inoculation of leaves or stems with infectious root sap but Allen (1948) showed that such inoculation did not cause big vein if the roots were protected from contamination with inoculum. Allen also showed that the infective agent retained infectivity in root juice diluted 1:200,0000 with water, and that, in soil or root juice, infection is destroyed by 30 minutes treatment at 65°c as well as by fumigation of infected soil with chloropicrin or dichloropropene-dichloropropane mixture. Dilution of one part of infected soil with 800 parts of autoclaved soil only slightly reduced disease incidence, while air-dried soil retained its infectivity for at least eight years (Pryor, 1946). An association of a tobacco-necrosis virus (TNV) with the big-vein symptom was noted by Fry (1952) and by Yarwood (1954) but transmission experiments of the former author were negative. Thus there is no conclusive evidence that the big-vein disease is caused by a virus.

Jagger, who had found big vein occurring in association with lettuce brown blight (Jagger and Chandler, 1934) later (Jagger, 1940) reported the common occurrence of *Asterocystis radicis* de Wildman (= *Olpidium brassicae* (Wor) Dang.) in plants affected by the latter

N.Z. J. agric. Res. 3: 301–304.

disease. Although suggesting the possibility, he did not regard this parasite as the cause of either brown blight or big-vein disease.

The following investigations were undertaken in an attempt to ascertain the cause of the big-vein disease.

Trials were carried out using a TNV isolated from lettuce roots and cultured in bean. Inoculum was poured round the roots of lettuce growing in four-inch pots and infection with TNV subsequently confirmed by back inoculation. Results given in Table 1 show that no big vein developed in the TNV-infected plants although symptoms appeared in plants inoculated with big-vein root sap.

TABLE 1. INOCULATION OF LETTUCE WITH TOBACCO-NECROSIS VIRUS AND BIG-VEIN ROOT SAP

Inoculum	Number of Plants Inoculated	Number of Plants Developing Big Vein
T.N.V.	76	0
Big-vein root sap	99	68
Water	182	1

Roots of lettuce plants from gardens where big vein occurs in Whangarei, Auckland, Wanganui, and Christchurch, when examined microscopically, were found to contain, in the epidermis, large numbers of thick-walled resting spores and zoosporangia of *O. brassicae*. No *Olpidium* could be found in roots of plants from two gardens in Palmerston North which were free from symptoms of big vein.

Infective lettuce root sap was centrifuged at 3000 rev/min for five minutes (MSE Major Centrifuge) when all the *Olpidium* spores were found in the sediment together with a little cell debris. The sediment was resuspended in water equal to the original volume and used in inoculation experiments. Table 2 gives these results, which show that all the big vein infection was in the sediment. If a virus was involved

TABLE 2. INCIDENCE OF BIG-VEIN SYMPTOMS IN LETTUCE PLANTS INOCULATED WITH FRACTIONS OBTAINED BY CENTRIFUGING BIG-VEIN ROOT SAP

Inoculum	Number of Plants Inoculated	Number of Plants Developing Big Vein
Sediment	56	28
Supernatant	56	0
Big-vein root sap	68	40
Water	63	0

it would be expected that infectivity would remain in the supernatant unless the virus was readily adsorbed on the spores or cell debris in the sediment.

Three plant species, *Veronica tournefortii* Gmel., *Stellaria media* (L) Vill., and *Spergula arvensis* L., occurring as weeds in big-vein lettuce crops were found to be hosts of *Olpidium*. Sap from the roots of these plants was poured round the roots of seedling lettuce. Big vein developed in some of the lettuce inoculated with sap from each species (Table 3).

TABLE 3. Production of Big-vein Symptoms in Lettuce Inoculated with Root Sap from Hosts of *O. brassicae*

Source of Inoculum	Number of Plants Inoculated	Number of Plants Developing Big Vein
Roots of *Veronica tournefortii*	22	3
Roots of *Stellaria media*	15	4
Roots of *Spergula arvensis*	10	2
Roots of big-vein lettuce	12	4
Water	21	0

In a trial carried out on a big-vein-infected commercial lettuce garden, fungicides were applied by watering on the soil seven days after lettuce had been planted. Plots treated with captan, Phygon, copper oxychloride, and thiram all had fewer big-vein plants than control at harvest time (Table 4).

TABLE 4. Incidence of Big Vein in Lettuce Grown in Infected Soil Treated with Fungicides*

Material	Per cent Infection at Maturity	Equivalent Angle**
Water	80.2	67.1
Copper oxychloride 15 lb/100 gal	59.5	50.3
Thiram 2 lb/100 gal	65.3	54.2
Phygon 1 lb/100 gal	54.2	47.5
Captan 2 lb/100 gal	43.4	41.7

---

* Applied by watering 3 gal per sq.yd on infected soil seven days after transplanting.

** Randomised block design used. Difference for significance 5% 10.8, 1% 15.2.

O. brassicae occurs in roots of lettuce growing in big-vein-infected soil. It was present in inocula which caused big vein, both the sediment after centrifuging and sap from roots of three weeds. A reduction in incidence of symptoms followed application of fungicides to infected soil. This evidence strongly suggests that *O. brassicae* is the cause of big-vein disease of lettuce.

Similar results have been obtained by Dr. R. G. Grogan and his co-workers in California. They have shown (Grogan *et al.* in press) that inoculation of lettuce with *Olpidium*-infected roots of lettuce and a number of other hosts resulted in big-vein disease. Inoculation with zoospore suspensions of *Olpidium* also produced big-vein symptoms.

ACKNOWLEDGEMENTS

The author wishes to thank the following: Miss J. M. Dingley of this laboratory for identifying the organism and for helpful discussions; Dr. R. G. Grogan, University of California for interesting correspondence, for comparing the *Olpidium brassicae* found in New Zealand with that in the U.S.A., and for access to his results before publication; Dr. H. R. Thompson, Applied Mathematics Laboratory, D.S.I.R., for design and analysis of experiments; and Messrs. R. H. Sherson and L. Lowe on whose properties field trials have been conducted.

REFERENCES

ALLEN, M. W. 1948: *Phytopathology 38:* 612-27.
DOOLITTLE, S. P.; THOMPSON, R. C. 1945: *Ibid. 35:* 484.
FRY, P. R. 1952: *N.Z. J. Sci. Tech. A34:* 224-5.
GROGAN, R. G.; ZINK, F. W.; HEWITT, W. B.; KIMBLE, K. A. (In press) *Phytopathology.*
JAGGER, I. C. 1940: *Ibid. 30:* 53-64.
JAGGER, I. C.; CHANDLER, N. 1934: *Ibid. 24:* 1253-6.
PRYOR, D. E. 1946: *Ibid. 36:* 264-72.
YARWOOD, C. E. 1954: *Plant Dis. Reptr 38:* 263.

THE TILLER POPULATION OF PASTURES

By K. J. MITCHELL and A. C. GLENDAY[1]

(*Received for publication, 21 January 1958*)

Summary

The reasons for examining the tiller population of grazed pastures are outlined; the procedures adopted are described and evaluated statistically; and examples are given to illustrate the information obtained for various types of pasture.

INTRODUCTION

In recent years there have been a number of studies of the rate and morphology of growth of individual plants of pasture species in various sets of environmental conditions (Mitchell 1955, 1956a, Langer 1956). There has also been some field examination of the habit of growth of various species. Results from this work suggest that in the majority of intensively grazed pastures in temperate zones the primary growth unit is the individual tiller[2], and that the same production per unit area of land can be produced by different species having a large number of small tillers or a smaller number of large tillers (Mitchell 1956b).

A pasture can be regarded as a population of such tiller units, each capable of growth and vegetative reproduction and with a finite life. As such it is subject to the normal dynamics of any population in which individuals of various species have to adjust themselves to varying environmental conditions, and to inter-relationships with individuals of other species in the same habitat. The amount of growth from a tiller, and its rate of vegetative reproduction, are determined not only by the availability of adequate mineral nutrients, light, and water, but also by the vigour and mechanical strength of its roots, by the placement of the tiller base at, above, or below the soil surface, by the physical crowding from other tillers, or by restrictions on the growth of new leaves and of axillary buds which may come from the presence of the sheaths of dead leaves around its base. Whether the life of an individual tiller is measured in weeks, months, or years, is dependent both on the species concerned and on general environmental conditions—including both climatic factors and management procedures. As with any population, the greatest vigour and productivity occur when the majority of individuals are relatively young. This can be achieved either by ploughing up and resowing a pasture or by adopting management practices which result in the continuing replacement of older tillers by vigorous young tillers.

If such views represent a reasonable background against which to consider the growth and potentialities of a pasture as a particular type of plant community, it is necessary to have methods of assessing

---

[1] Grasslands Division and Applied Mathematics Laboratory respectively, Department of Scientific and Industrial Research, Palmerston North.

[2] For simplicity of terminology all types of live shoots in a pasture are referred to as tillers.

N.Z. J. agric. Res. 3: 305-318.

botanical composition which will give information bearing directly on these problems.

Many different techniques of botanical analysis of pastures have been used to varying extents. In the era when pastures were regarded in large measure as botanical communities whose floristic characteristics were adapted to the environment, and little under the conscious control of man, plant sociological techniques mostly modelled after those of Braun-Blanquet were generally used. These were concerned primarily with assessing the number of species present and the evenness of their distribution across an area of land. Later, with the selection of strains of pasture species with agronomically desirable production and survival characteristics, and with the development of techniques for the replacement of established pastures by simple mixtures of these new strains. interest in examination of floristic characteristics was replaced by emphasis on the determination of the proportion of desirable species present. For this purpose, two main methods of botanical analysis were used. Firstly, separation and weighing of herbage of different species cut from an area of pasture to give a direct indication of the relative yields of the component species. Secondly, point-analysis techniques (Levy and Madden, 1933), which state the proportion of the pasture cover contributed by each of the species. The latter gives results affected by the height of the pasture cover, by whether or not there has been any recent grazing, and by the form of growth of individual leaves of different species, but it serves very well where interest is centred on a few species.

However, neither of the above types of technique provide the information required where the subject for study is the population of tillers of various species, and the extent to which the tillers make full use of light. For this, it is necessary to make an estimate not only of the relative numbers of tillers present, but also of the numbers per unit area of ground.

Although the botanical assessment of a pasture in terms of numbers and area distribution of individual tillers does represent a departure from the lines of botanical analysis which have predominated during recent decades, this approach has been considered by a number of workers in the past. Armstrong (1907) counted numbers of individual plant units in pastures using criteria for individual units similar to those adopted in this work. Similar work was subsequently carried out by other investigators, e.g. Yapp, Johns, and Jones (1917). Davies (1933). In Britain the sampling unit for pasture was generally a six-inch square. Tillers were either counted *in situ* or a sample of turf was cut out and taken to a laboratory for counting. For samples of this size, counting of tiller numbers in a turf is both tedious and difficult.

In the late 1930s de Vries developed the 25-sq.cm "plant sociological method" for botanical analysis of pastures (de Vries, 1938). In this, samples were taken at regular intervals across the pasture either by cutting herbage in the field or, in the case of short pastures, by removing 25-sq.cm plugs which were taken back to the laboratory for examination. De Vries preferred not to determine the numbers of tillers of various species in each plug. Such information was thought to be much more laborious to obtain than a direct weight analysis of the sample, and it was also considered that because of large differences in size between tillers of various species, it would give little information concerning the potential production of various species (de Vries 1949).

Measurement of the production from individual tillers under a range of environmental conditions (e.g. Mitchell 1956) made it possible to overcome the second disadvantage, and the problem became one of determining whether a suitable flexible and speedy method could be developed for assessments of tiller numbers in pasture. The preference for tiller counting instead of weight estimates was reinforced by the view that the relative contribution of species of considerably different growth habits to a weight sample may be influenced by the height of a pasture at time of sampling, by whether it has been grazed recently, and by whether it is normally grazed continuously or intermittently. If the individual tiller is taken as the unit, the results of an assessment of a pasture's botanical composition should not be directly influenced by such factors.

This paper describes the development of the plug sampling technique now used to obtain the tiller data required.

METHODS

Plug Size

Biological, statistical, and physical factors had to be considered in determining a suitable size of plug for general use.

It was considered important that the area of each plug should represent a biological unit for tillers in a pasture. This means an area which could become dominated by a species under appropriate management if one or more tillers are present. The percentage of such unit areas which is occupied by a species gives a measure of its potential dominance in a pasture. The size of such an area will vary according to the habit of growth of individual species. As perennial ryegrass (*Lolium perenne*) is the most important grass species in the majority of high-producing pastures in New Zealand, the plug area was considered primarily in relation to the habit of growth of this species.

From the point of view of statistical efficiency, the optimum plug size would vary with species, season, sparseness, and patchiness of stand, and no overall most efficient size could be determined. For these reasons it was decided that a prior trial of the statistical efficiency of plug size was not warranted.

Physical factors were also of importance. Larger plug sizes increase the difficulty of handling plugs owing to breakage and a decline in accuracy and speed of tiller counting where there are large numbers in each plug. On the other hand, smaller plugs contain a higher proportion of edge area where tillers are damaged, and there may be crumbling of the soil and loss of tillers in handling. This could give some bias if varying with season. However, even in relatively small plugs such difficulties did not become of major importance, for tillers were counted on the soil surface of the plugs, and apart from the minor exception of aerial rooting, each tiller had, by definition, to have roots of its own present within the plug. Such counting procedures also meant that severing of tillers when sampling a lax pasture did not lead to bias in the data by comparison with short pastures.

After field tests had been carried out, a circular quadrat of 2 inches in diameter (giving an area of 20.3 sq.cm) was adopted as the standard size.

The Sampling Tool

Fig. 1 shows the pasture sampler, and Fig. 2 gives details of its construction.

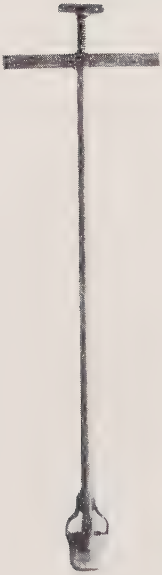


Fig. 1.

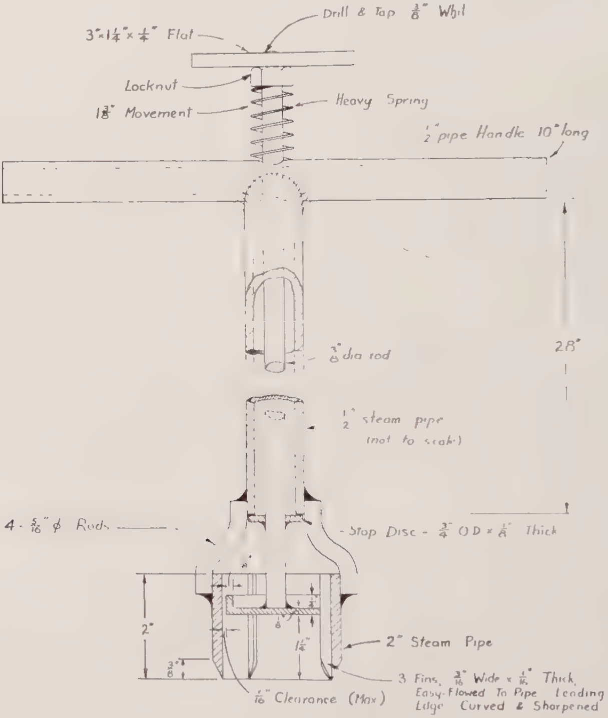

Drill & Tap $\frac{3}{8}$" Wht
3"×1¼"×¼" Flat
Locknut
1⅜" Movement
Heavy Spring
½" pipe Handle 10" long
⅜" dia rod
28"
½" steam pipe (not to scale)
4 - $\frac{5}{16}$" ϕ Rods
Stop Disc - ¾" OD × ⅛" Thick
2"
⅛
¼
1¼"
⅜"
2" Steam Pipe
$\frac{1}{16}$" Clearance (Max)
3 Fins, $\frac{3}{16}$" Wide × $\frac{1}{16}$" Thick, Easy-Flowed To Pipe Leading Edge Curved & Sharpened

Fig. 2.

Fig. 1.—Pasture sampler.
Fig. 2.—Constructional drawing of pasture sampler.

In use, the head of the sampler is pushed or thrown into the soil with sufficient force to allow a penetration of 1–1¼ inches. The cross arm at the top of the handle is then given a twist to shear off the plug from the soil below, and the sampler lifted out. To remove the plug, the sampler is inverted and the plate at the top of the ejector rod tapped firmly on the ground to eject the plug. Plugs are then placed sequentially in a 2½-inch diameter holding tube, and taken to a herbage laboratory where the numbers of tillers in each plug are recorded.

Sampling Procedure

The most efficient method statistically in this type of work is "stratified random sampling", in which the field is divided into any obvious sub-areas and the number of random samples taken from each is made proportional to its area (Hendricks 1948). In its ultimate form, the field is divided into as many equal-sized areas as there are samples to be taken, one sample being randomly located within each area.

The method ensures that if n samples are to be taken, no area of $\frac{1}{n}$ of the total remains unsampled, thus avoiding bias because of uneven sampling of the field. It also allows field maps to be drawn from the samples collected systematically, and trends may be removed from the data on analysis.

The disadvantage of the method is the difficulty of dividing irregularly shaped fields into equal areas, and the location of random sites in each.

Theoretically, there is no objection to equally spaced samples, provided the spacing is made sufficient to ensure independence between sites, and this is the system used in sampling spaced row crops, such as potatoes (Wishart 1934). If the spacing is sufficient to ensure independence, this spacing need not be accurately determined, and a regular number of ordinary paces is quite sufficient.

Hence the method can be imitated simply, and its advantages retained, in the following manner. Suppose 100 plugs are to be taken from an area of 1 acre (4840 sq.yd). The observer walks up and down the field on lines 7 yards apart to cover the field, and takes a sample every 7 yards along these lines. Thus he samples once in every 49 sq.yd, as required. Generalising the argument, the correct spacing between lines, and between samples along the line, will be $\sqrt{4840\ A/n}$ yards, where A is the area in acres, and n is the number of samples required, the calculation and method being independent of the shape of the field.

The method as applied, which may be termed "systematic line sampling", is similar to that of de Vries (see Nielen and Dirven, 1950), in which lines parallel to both diagonals of the field are traversed.

Precautions are taken so that the observer does not unconsciously select or avoid specific sampling sites. The usual method is for the observer to take the required number of paces and sample blindly at arm's length to right or left. Only if the field sampled is stony or excessively fouled is it necessary to locate the intended site by means of a rod before using the sampler to take the plug. If impossible sites are chosen, these must be recorded, and due allowance made in the estimates of cover, average tiller counts and percentage occurrence.

RESULTS

Statistical

Calculation of Standard Errors of Mean Tiller Counts and Occurrence Percentages, and Estimation of Sampling Densities Required

If the data obtained were normally distributed there would be no difficulty in calculating coefficients of variation from preliminary runs, and from these giving accurate estimates of the numbers of samples required to give any stated precision. However, it was realised that many different distributions would be met, and that an overall simple system, provided it was reasonably free from bias, would be necessary rather than a rigorous examination of each set of data. For this reason, standard errors of tiller counts and estimates of plug numbers required were calculated in the ordinary way as though the data were normal. That the procedure does not lead to large errors is shown in Table 1, in which estimates based on a per-plug analysis are compared with those based on a per-16-plug analysis.

TABLE 1. COEFFICIENTS OF VARIATION OF TILLER NUMBER PER PLUG AND NUMBERS OF PLUGS REQUIRED PER PASTURE

Species	Plugs per Sample	Sheep Pasture (1)		Sheep Pasture (2)		New Dairy Pasture		Old Dairy Pasture	
		v[1]	n[2]	v	n	v	n	v	n
Perennial Ryegrass	1	50	25	114	131	78	61	137	186
	16	12	22	30	142	19	55	32	167
White Clover	1	69	48	136	184	195	378	78	61
	16	16	41	36	205	43	299	19	57

[1] v = Coefficient of variation (%) per 1 or 16 plug total.

[2] n = No. of original plugs required for SEs of mean tiller number per plug of ± 10%.

This was done in the following manner. From the sets of counts of different species in a variety of pasture types (the same pastures recorded in Fig. 3) random sets of 16 were drawn and the totals per 16 plugs examined. Clearly the distributions of 16-fold totals would be much closer to normal, and in most cases the truncation removed. Then if these new counts could be regarded as the distribution of counts to be expected from plugs of four times the diameter, estimates of the number of such imaginary plugs could be compared with the original estimates. Differences in the estimates of the total area of pasture required for dissection would demonstrate invalidity of ignoring non-normality in making the original calculations.

It will be seen that skewness and truncation have had no serious effect on the validity of the estimates made in the usual way. The method is most open to objection where the number of zeros in a set of data is great.

For standard errors of "percentage presence" or "percentage absence" the simple binomial model may be used, assuming that a species can be present or absent only, with no gradation in either class. The standard errors of the percentages so obtained are simply

$$\frac{(\%\ \text{presence})\ (\%\ \text{absence})}{(\text{number of plugs})-1}$$

Note that the percentage absence can be read directly from the first columns of Fig. 3.

Tiller Distributions

It will be seen that the method of presentation of the distributions in Fig. 3 has been to condense counts into the following classes: 0, 1-2, 3-6, 7-14, 15-30, 31-62, 63 +, that is, the number of counts per class form a geometrical progression 1, 2, 4, 8, 16, etc. This has the effect of very largely removing the skewness of the distributions, the effect being similar to that of plotting counts on a logarithmic scale. Thus data which originally were log-normally distributed would appear to be normal under this system.

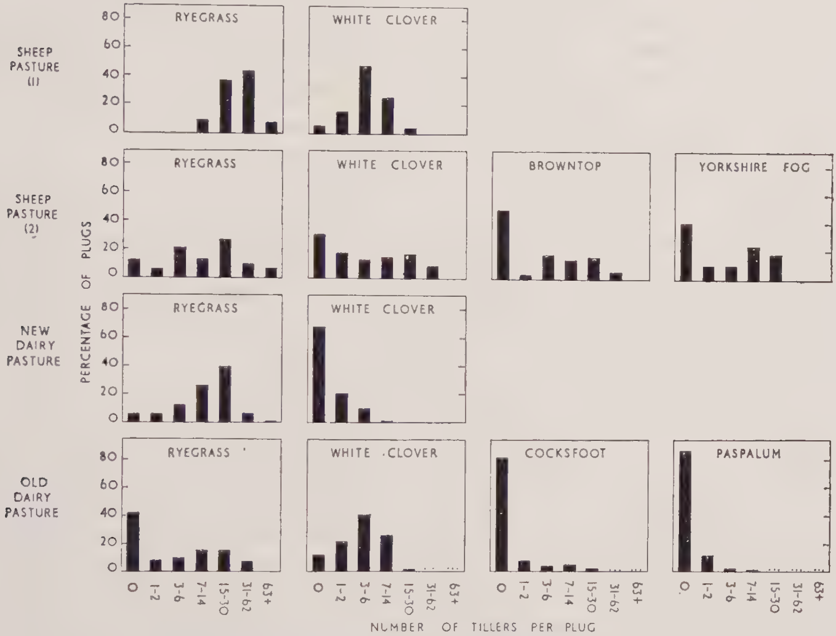


Fig. 3.—Numbers of tillers per plug grouped according to frequency of occurrence. Data are presented for (1) A sheep pasture of ryegrass and white clover, sown 1929. (2) An old sheep pasture containing a mixture of species and sown over 30 years ago. (3) A dairy pasture of short-rotation ryegrass and white and red clovers sown the previous autumn. (4) A dairy pasture over 30 years old.

Besides varying degrees of skewness, there are also varying degrees of range in relation to mean, indicating clumping of the counts, and of truncation, where the zero class is more frequent than any other.

The coefficients of variation of counts per plug varied with species, and with age and type of pasture, as shown in the preceding table. It will be seen that they are rarely less than 100%. With a coefficient of variation of 100%, 100 plugs per pasture would be required to determine mean tiller counts with a standard error of ± 10% and percentage presence with a standard error of 5% or less (depending on the percentage presence itself). Generally the aim has been to measure pastures with an accuracy at or approaching this level.

Although no tests of significance are quoted in the text, standard errors are given, and any comparisons of mean tiller counts required can be made by the usual procedure of calculating the weighted mean variance and applying a "t" test, or by the following simple rule. If the number of plugs examined in two sites being compared are approximately equal and greater than 30, as was always the case in the data presented, then a significant difference at the 5% level of probability will be approximately

$$2\sqrt{E_1^2 + E_2^2}$$

where E_1 and E_2 are the quoted standard errors of the means.

Indices of Clumping

These were applied but the results are not given in detail. The usual indices (Moore 1953; David and Moore 1954) use the Poisson distribution (postulating random occurrence of individual tillers or nodes) as a criterion and test departures from this. For example

$$I = \frac{(\text{number of plugs}) \times (\text{variance})}{(\text{mean})}$$

gives t = 18.5 and 34.6 for ∞ d.f. for ryegrass in Pasture 1 and 2 respectively of Fig. 3. However, examination of the two distributions quoted shows quite clearly that the distribution of ryegrass in Pasture 1 is very narrow, in view of the high mean count, with few small or very large counts (i.e. non-clumped), while that in Pasture 2 is very wide in relation to the mean count, with many small and large counts (i.e. clumped). The usual indices of clumping did not show this, indicating the inadequacy of the Poisson distribution as a test criterion. If the log-normal distribution is used as a criterion (by applying the chi-sq. test of goodness of fit to the normal distribution fitted to the distributions classified logarithmically as shown), the results are as follows: for Pasture 1 chi-sq. = 0.45 for 1 d.f. ($p = 0.50$) showing that there is no significant departure from the log-normal model, while in Pasture 2 chi-sq. = 9.15 for 3 d.f. ($p = 0.04$), showing significant clumping as compared with a log-normal distribution.

It should be noted that in order to distinguish between clumping (as in single ryegrass plants in a sparse stand) and patchiness (as often found in weed infestation) tiller counts from a small sampler are inadequate unless the position of each plug in a field is recorded and individual plugs taken sufficiently close together. In that case, vegetation mapping can be carried out.

Assessments of Botanical Composition of Pastures

Although the purpose of this paper is to describe a technique of botanical analysis rather than to consider the implications of the results of various studies for which it has been used, it is appropriate to give a series of examples to illustrate the type of information that has been obtained.

Different Types of Pastures

Table 2 shows the tiller numbers per square foot and the percentage presence, for all species in four pastures, all of which would

TABLE 2. TILLER NUMBER PER SQUARE FOOT AND PERCENTAGE OF PLUGS CONTAINING VARIOUS SPECIES

Species	Sheep Pasture (1)		Sheep Pasture (2)		New Dairy Pasture		Old Dairy Pasture	
	Tiller Number	% Presence	Tiller Number	% Presence	Tiller Number	% Presence	Tiller Number	% Presence
Ryegrass (*Lolium* spp.)	1530	100	851	87	700	94	400	57
Browntop (*Agrostis tenuis*)			345	53				
Cocksfoot (*Dactylis glomerata*)			106	28	1	2	55	19
Yorkshire fog (*Holcus lanatus*)	18	16	268	60	23	5	14	5
Paspalum (*Paspalum dilatatum*)							14	14
Poa annua and *Poa trivialis*	4	4	846	73	23	14	97	27
Poa pratensis			97	43			60	35
Dogstail (*Cynosurus cristatus*)	4	2	37	10				
Goose grass (*Holcus mollis*)			5	2				
Chewing's fescue (*Festuca rubra* var. *commutata*)			23	8				
Sweet vernal (*Anthoxanthum odoratum*)			147	32				
White clover (*Trifolium repens*)	258	94	432	72	34	32	212	88
Red clover (*Trifolium pratense*)					14	14		
Lotus major (*Lotus uliginosus*)					4	1		
Suckling clover (*Trifolium dubium*)					9	9		
Weeds			46	20	23	35	5	7
Total tillers	1814		3203		831		857	

be considered high-producing pastures as judged by stock-carrying capacity. The frequency distributions of the main species in each pasture are shown in Fig. 3.

Tiller density, recorded as numbers per square foot, is much greater in the sheep than in the dairy pastures. This is probably largely the result of the closer and more continuous grazing normally occurring on a sheep pasture. In the more recently sown pastures, ryegrass and white clover are the dominant species, but in the older pastures, both of which were established well over 30 years ago, other grass species are important constituents of the sward. This is particularly so in the sheep pasture where browntop, Yorkshire fog and meadow grasses (*Poa* species) are all prominent.

The dense and even cover of ryegrass in the more recently sown of the two sheep pastures, and also in the new dairy pasture, is well shown by the frequency distributions for these pastures. In very few plugs is ryegrass absent, and the majority of the plugs have over ten tillers per plug of 20 sq.cm. The ryegrass cover is becoming rather more irregular in the old sheep pasture and is noticeably sparser in the old dairy pasture. In the latter, nearly fifty per cent of the plugs have no ryegrass present, and many of the other plugs have only a few tillers each.

The change in pattern of distribution of white clover between the first spring when it is just becoming established and later years when it develops an even and relatively dense spread through the pasture can be seen by comparing the new dairy pasture with the other pastures. The former had been sown down only six months before, that is, in the previous autumn.

Absence of a species from a high proportion of the plugs may be due to its tending to grow in clumps, or to its being evenly but sparsely spread over the field. Where a fairly high proportion of those plugs in which the species is present give high tiller counts, it is an indication of clumping, but where the majority of the plugs in which the species is present give low tiller counts it is an indication of a sparse but even distribution; cf. paspalum and cocksfoot in the old dairy pasture.

Seasonal Trends Within a Pasture and Responses to Management

These are being followed in a number of different types of pastures. As a dairy pasture of certified N.Z. short-rotation ryegrass and white clover may show considerable seasonal changes in the balance between the two species, data for a pasture of this type are presented in Fig. 4. This pasture was initially sown down in May 1955. The essential features of the changes are: In the first spring after sowing there was an even and relatively dense cover of ryegrass and an even but still fairly sparse cover of white clover. During the following summer and autumn the number of tillers per square foot of white clover rose considerably and it developed a very even spread over the pasture, being present in about 90% of the plugs. Subsequently this even spread over the pasture was maintained but there was seasonal variation in the average number of tillers per square foot.

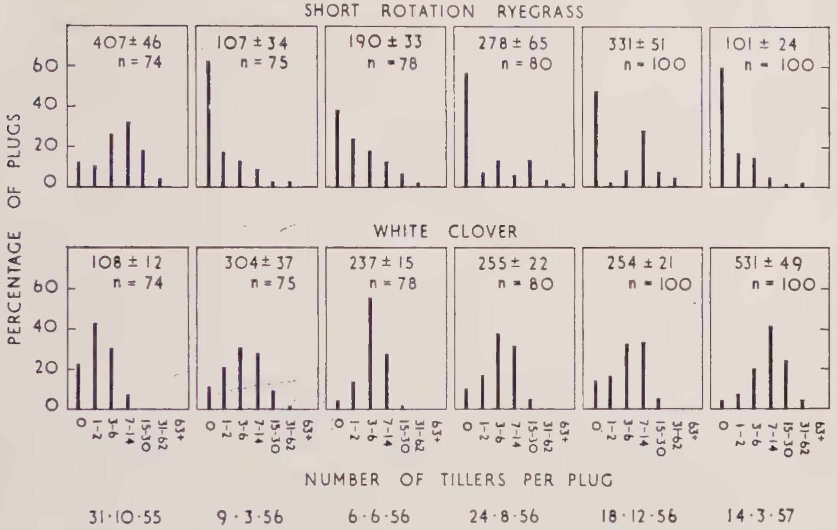


Fig. 4.—Changes in tiller density and pattern of distribution of short-rotation ryegrass and white clover in a newly sown pasture. (Mean tiller number per square foot and its standard error are given on each diagram).

In the short-rotation ryegrass the history was different. Although there had been a relatively even and dense cover of tillers in the spring, this showed substantial changes after the first spring when the short-rotation ryegrass became irregularly distributed over the area and there was a large decrease in average number of tillers per square foot.

The effects of an oversowing in April were apparent in the June sampling, both as an increase in the proportion of plugs with ryegrass present and as an increase in number of plugs with a few tillers present. The subsequent reversal of these trends, as shown in the sampling in late August, suggests that many of the small newly established plants failed to survive the spring grazings.

A considerable amount of clumping is often seen in older dairy pastures in which short-rotation ryegrass is the dominant grass species. The development towards this clumping can be deduced from the frequency distributions. If the reversal of trends resulting from the April oversowing is ignored, it can be seen that there was first a substantial rise in proportion of plugs without ryegrass tillers. Then among the plugs that did contain ryegrass tillers there was subsequently an increase in the proportion of plugs containing a large number of tillers. This trend continued until December 1956. However, the subsequent sampling in March showed a considerable decline in number of tillers per plug, presumably owing to the warm dry conditions of that summer.

Trends Within a Pasture

The data presented in the preceding sections were from pastures in which there were no obvious trends in species distribution. Where these occur, and are complex, they can be examined by using detailed techniques such as the method described by Pidgeon and Ashby (1942). If there are unidirectional gradients across a field either a transect along the gradient with sampling at close intervals, or a series of samplings each made across the gradient, can be used. Both have been tried, and results for two adjacent hill pastures, one on a sunny face and the other on a shady face, are presented (Fig. 5 and Table 3).

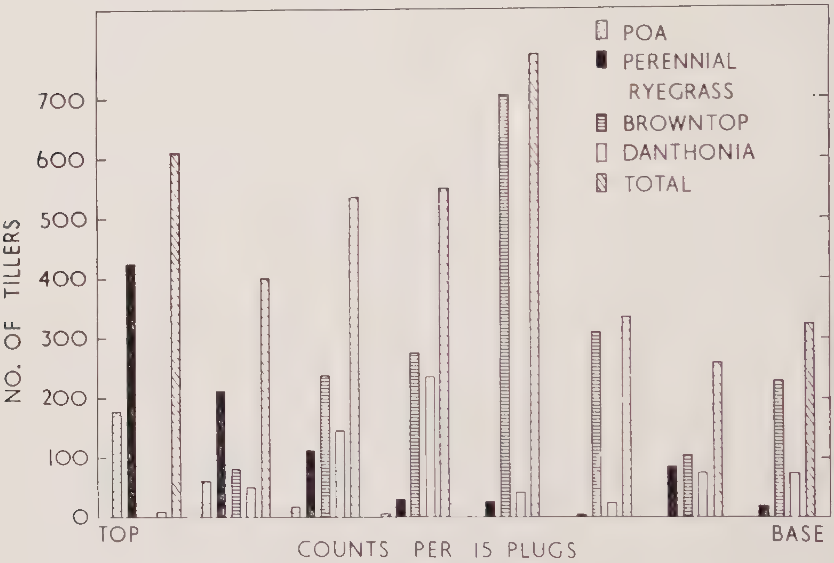


Fig. 5.—Trends along a transect from the ridge to the valley base of a sunny hill pasture. A plug was taken every five feet.

In the sunny face (Fig. 5), ryegrass and meadow grass (*Poa* spp.) were the dominant species at the top where fertility was high owing to concentration of sheep at night. These species were replaced by *Danthonia* and browntop as the fertility declined lower down the slope. Most *Danthonia* occurred near the top of the slope where it was drier and sunnier and browntop reached greatest density lower down where the slope was cooler and moister. Over the bottom third the transect passed down an old slip. There the pasture cover became sparser with browntop dominant in the wetter areas and *Danthonia* dominant in dry. An occasional patch of ryegrass occurred.

On the shady face (Table 3), total tiller density was greatest at the crest where the slope was not great and sheep camped at night. The tiller density dropped at the head of the slope where the transect crossed the raw soil of the tops of several slips, increased across the centre of the slope, and finally became least at the bottom of the slope where there was much boggy ground, including wash from the slips above. The density of ryegrass declined from the top to the bottom of the slope, but white clover showed a much more even spread and was densest in the lower portions of the slope. Yorkshire fog on one hand, and

TABLE 3. PASTURE COMPOSITION AT VARIOUS LEVELS ON A SHADY FACE

Position	Ryegrass	Browntop	Chewings fescue	Sweet vernal	Yorkshire fog	White clover	Total Tillers
Crest	740	630	16	92	120	141	1950
Top of slope	305	365	38	61	75	90	1060
Mid slope	210	630	210	165	10	180	1630
Lower slope	144	330	190	295	—	325	1410
Base	42	335	82	36	82	127	990
Standard[1] Error (%)	24	21	38	31	35	30	10

[1] Coeffs. of variation were relatively constant over the slope for each species; Standard errors are therefore given as percentages of the quoted figures in each column.

Chewing's fescue and sweet vernal on the other hand, offer an interesting contrast. The former was present at the top and bottom of the slope but almost absent from the centre of it; but the latter two species had highest tiller densities at the centre of the slope.

CONCLUSION

This paper is intended to decribe the procedures being used for the examination of pastures, the reasons for their adoption, and to illustrate the types of results being obtained. Accordingly, detailed discussion of ecological and agronomic implications of the examples presented will be reserved until a full report is made of the various investigations from which these examples are drawn.

The botanical analysis procedures described have now been in use for several years and have been adopted by workers concerned with a number of different aspects of pasture growth. It has been found that sampling of pastures can be carried out by workers without technical training, samples can be transported over long distances provided this takes no more than a day or two, and they can be held for a week or more in cold rooms without deterioration. Sampling can be done at any time except when the soil is very soft or very dry and friable, and with two persons at work takes approximately half an hour to collect 100 plugs, and up to an hour to carry out the tiller counting in a herbage laboratory.

ACKNOWLEDGEMENTS

Mr. I. A. E. Atkinson participated in the initial trials to develop the technique, and Miss D. P. Owen assisted with the development and has supervised the dissection of very many plugs. Their assistance is gratefully acknowledged.

The authors thank Dr. P. D. Sears and Messrs. F. E. T. Suckling, E. P. Neilsen, W. J. Diprose, D. R. Willis, and A. J. Harris for assistance with selection and sampling of pastures.

REFERENCES

ARMSTRONG, S. F. 1907: The Botanical and Chemical Composition of the Herbage of Pastures and Meadows. *J. agric. Sci. 2:* 283.

DAVID, F. N.; MOORE, P. G. 1954: Notes on Contagious Distributions in Plant Populations. *Ann. Bot. n.s. 18:* 47.

DAVIES, W. 1933: Methods of Pasture Analysis, VI. The Percentage Tiller Estimation Method. *Agric. Progr. 10:* 242.

DE VRIES, D. M. 1938: The Plant Sociological Combined Specific Frequency and Order Method. *Chron. bot. 4:* 115.

——————1949: Survey of Methods of Botanical Analysis of Grassland. *Proc. 5th int. Grassl. Congr.:* 143.

HENDRICKS, W. A. 1948: Mathematics of Sampling. *Virginia Agric. Exp. Sta. Special Tech. Bull.*

LANGER, R. H. M. 1956: Growth and Nutrition of Timothy, I. The Life History of Individual Tillers. *Ann. appl. Biol. 44:* 166.

LEVY, E. B.; MADDEN, E. A. 1933: The Point Method of Pasture Analysis. *N.Z. J. Agric. 46:* 267.

MITCHELL, K. J. 1955: Growth of Pasture Species, III. White Clover, Subterranean Clover and Lotus Major. *N.Z. J. Sci. Tech. A37:* 395.

——————1956a: Growth of Pasture Species under Controlled Environment, I. Growth at Various Levels of Constant Temperature. *N.Z. J. Sci. Tech. A38:* 203.

——————1956b: Influence of Light and Temperature on the Growth of Pasture Species. *Proc. 7th int. Grassl. Congr.:* 58.

MOORE, P. G. 1953: A Test for Non-randomness in Plant Populations. *Ann. Bot. n.s. 17:* 57.

NIELEN, G. C.; DIRVEN, J. G. 1950: The Accuracy of the 25 cm^2 Specific Frequency Method. *Versl. landbouwk Onderz. 56:* 13.

PIDGEON, I. M.; ASHBY, E. 1942: A New Quantitative Method of Analysis of Plant Communities. *Aust. J. Sci. 5:* 19.

WISHART, J. 1934: Statistics in Agricultural Research. *Supp. J. Roy. stat. Soc. 1:* 26.

YAPP, R. H.; JOHNS, D.; JONES, O. T. 1917: The Salt Marshes of the Dovey Estuary, II. The Salt Marshes. *J. Ecol. 5:* 65.

THE INFLUENCE OF TREADING ON PASTURE
A PRELIMINARY STUDY

By D. B. Edmond, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 20 December 1957*)

Summary

A technique for the study of treading on pasture was tested and found to be satisfactory.

A ryegrass/clover pasture on Manawatu silt loam of good structure, and mature Romney sheep, were used during the winter and spring 1956.

Treading damaged the pasture; most of the effect followed the first treading but repeated treadings altered the botanical composition, white clover being susceptible. Increased treading had increased influence on plant and soil.

INTRODUCTION

New Zealand pastoral farming systems are based on year-long grazing at relatively high stocking rates. The amount of treading can be large (McCulloch and Connell 1934). Over the years, Levy, in particular, has spoken of the general importance of treading, and in 1926 he reported that treading helped to improve hill pasture production by destroying weed species. There is a number of unresolved problems, such as the controversy centred about the rotational grazing and set-stocking systems, which might become more simple if the effects of treading are understood.

In general, the broader aspects of the plant-animal-soil relationship have been studied, but Bates (1930, 1935, 1951), Chandler (1940), Crocker (1953), Davies (1938), Klecka (1937), Kudelin (1952), Levy (1926), Lieth (1954), and Steinbrenner (1951) have observed that treading can damage pasture species. Bruising and destruction of plants (Bates, Davies, Klecka, Levy, and Lieth), and compaction and puddling of the soil (Bates, Klecka, Kudelin, Lieth, and Steinbrenner) have been specially noted. Treading was seen to alter pasture composition. Thus the immediate effects of direct injury to plant and soil should be distinguished from the persistent effects of altered botanical composition and changed soil condition, physical and chemical.

There was no known systematic investigation of treading. However, O'Connor (in press) has since reported briefly on some aspects of the subject. It seemed necessary to study treading as a single factor free from the effect of defoliation and soil fertility. This paper reports the testing of a technique for studying short-term effects of treading and its application to the determination of critical treading intensities.

METHOD

A uniform pasture was used, consisting of New Zealand Certified short-rotation ryegrass (*Lolium perenne* L. × *L. multiflorum* Lam.)

and New Zealand Certified white clover (*Trifolium repens* L.), growing on Manawatu silt loam of good structure and high fertility, at Palmerston North.

Treading treatments were applied by driving mobs of mature Romney sheep along narrow fenced plots (Fig. 1.) The animal effect was confined to treading by preventing defoliation and excretion, the herbage being removed for measurement before each treading and the sheep being underfed for 12–24 hours before treatment.

In the absence of pertinent literature, it was assumed that a sheep walked about 1.7 miles per day. An area 1.7 miles long and 4 ft 10 in. wide is one acre, and thus, by using a plot effectively of this width, the passage of one sheep imposed a treading treatment equivalent to that of one sheep grazing one acre for one day. Thus any grazing rate (quoted as a stocking rate equivalent of *n* sheep/acre) could be imitated by walking *nd* sheep once along the plots every *d* days. For example, the treading of 10 sheep/acre could be imitated by walking 300 sheep along the plots once every 30 days, or, as was done in practice, to obtain equal treading in both directions and to limit mobs to manageable sizes, by walking 30 sheep 5 times in each direction.

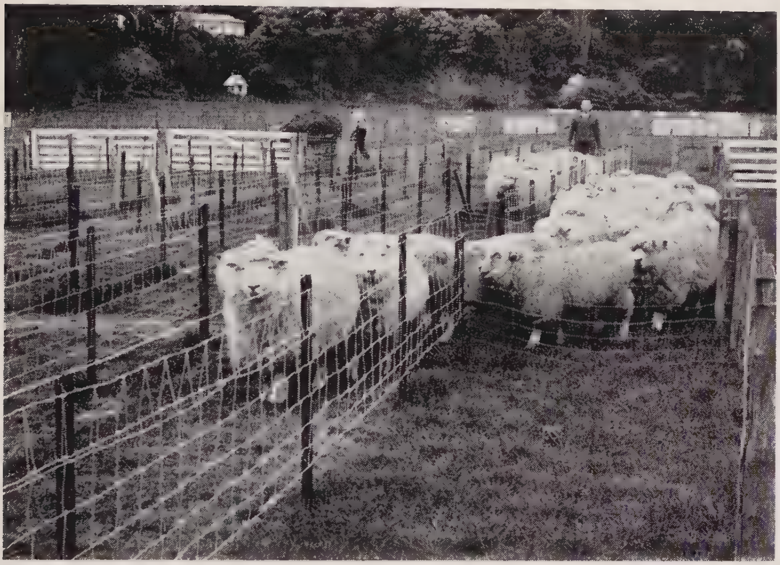
The treatments, replicated five times, were applied when soil moisture was near field capacity (about 40% for top 3 in.) which necessitated different time intervals. The treatments, dates of application, and sheep passages per plot (each 55 ft long) are shown in Table 1.

TABLE 1. SHEEP PASSAGES PER PLOT

Treatment (Stocking-rate equivalent, sheep/acre)	31 July (*)	28 August (28 days)	4 October (37 days)	19 October (15 days)
0	0	0	0	0
4	120	112	148	60
8	240	224	296	120
12	360	336	444	180
16	480	448	592	240
20	600	560	740	300

* Assumed that 30 days had elapsed since previous treading.

Immediately before treading, herbage yields were determined from a 2 ft strip cut with a rotary mower set at 1 in. height. For tiller and node counts, 25 randomly sited plugs (total area 78 sq.in.) were taken from each plot (Mitchell and Glenday 1958), and for soil-moisture determinations, 4 plugs per plot were taken ($\frac{1}{2}$–2 in. depth). After the second treading (28 August), soil volume weights were secured from each of four equal depths down to 6 cm, with five random samples (1.5 cm diam.) per plot. Samples showing worm holes were discarded. Late in January 1957, the untrodden and the 20-sheep plots were sampled for soil moisture under dried soil conditions, and for root yield using two 3-sq.in. samples per plot at three depths ($\frac{1}{2}$–2, 2–6, 6–10 in.). After



—*Photo: H. Drake.*

Fig. 1.—Romney sheep being driven along narrow fenced plots.

the last treading (19 October) and mowing (1 November) the area was closed. On 25 January 1957 the ryegrass seedheads were counted in all plots using two 1-sq.yd samples per plot.

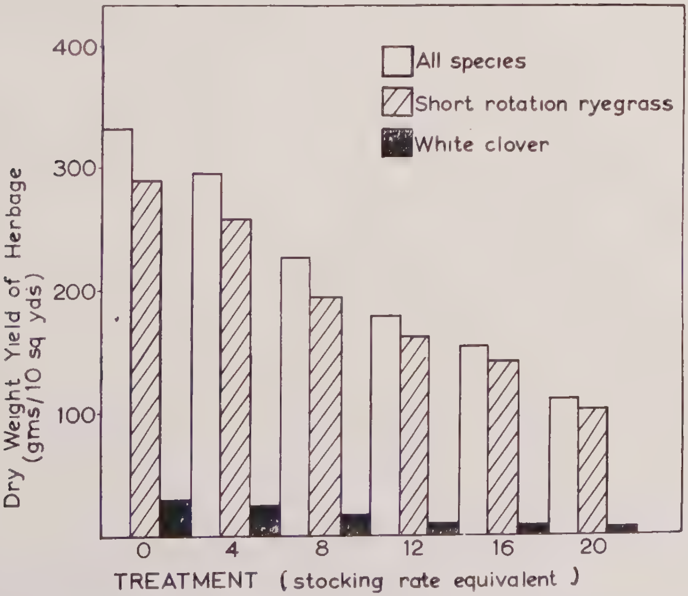


Fig. 2.—Some effects of sheep-treading treatments on a uniform pasture, as measured 28 days after treatment.

TABLE 2. HERBAGE YIELDS (Dry Wt.—lb/acre)

Treatment (Stocking-rate equivalent)	All Species				Short-rotation Ryegrass				White Clover			
	28 Aug.	4 Oct.	19 Oct.	1 Nov.	28 Aug.	4 Oct.	19 Oct.	1 Nov.	28 Aug.	4 Oct.	19 Oct.	1 Nov.
0	355	1890	212	301	304	1440	181	235	28	285	20	49
4	313	1620	173	266	273	1277	150	217	23	211	18	38
8	242	1589	124	250	210	1287	110	214	18	174	10	27
12	193	1174	131	207	170	949	122	181	13	134	7	13
16	171	1264	73	192	149	1055	68	173	11	113	2	13
20	121	1042	73	173	111	922	68	162	5	63	1	6
S.E. ±	18	60	7	11	17	64	6	11	1.8	21	2.6	2.9
Sig. diff. at 5% level	54	177	31	30	51	189	19	32	5.4	64	7.7	8.6

RESULTS

Herbage Yield

Increases in treading produced significant and progressive reductions in yield of all species (Table 2). Regression analyses (not shown) failed to show noteworthy departures from a linear relationship between treading rate and yield. Most of the effect was produced by the first treading, results of which can be seen in Fig. 2.

Tiller and Node Numbers

Ryegrass tillers and white-clover nodes were reduced significantly and progressively by increasing intensities of treading, with the exception of ryegrass on 4 October and white clover on 28 August (Table 3). The regression analyses (not shown) showed only one case, white clover on 28 August, where there was a significant departure from a linear relationship between treading rate and number of tillers or nodes. With both species, much of the effect occurred at the first treading, but in white clover it became more pronounced with further treadings. *Poa* spp. were affected in the same way as short-rotation ryegrass.

TABLE 3. TILLERS AND NODES (No. per sq.ft)

Treatment (Stocking-rate equivalent)	Ryegrass Tillers			White Clover Nodes		
	28 Aug.	4 Oct.	19 Oct.	28 Aug.	4 Oct.	19 Oct.
0	292	209	295	159	126	109
4	294	177	240	124	131	124
8	266	233	295	120	146	102
12	271	205	262	105	138	72
16	216	214	229	127	127	72
20	201	275	246	116	114	59
S.E. ±	28	24	22	11	13	13
Sig. diff. at 5% level	83	72	66	31	39	39

Root Yield

There were no significant differences between treatments (Table 4).

TABLE 4. ROOT YIELDS (Dry Wt.—g/100 cm^3 soil)

Treatment (Stocking-rate equivalent)	Depths			
	½–2 in.	2–6 in.	6–10 in.	Total (½–10 in.)
0	2.95	1.03	0.27	4.25
20	2.42	0.58	0.16	3.16
S.E. ±	0.39	0.19	0.04	0.57

Soil Moisture

On 19 October there were significant differences between treatments, increases in treading being associated with less soil moisture (Table 5). In dry conditions, 30 January 1957, the untrodden plots held more soil moisture than did the 20-sheep plots (sig. at 10% level only).

TABLE 5. SOIL MOISTURE (% dry soil, ½-2 in. level)

Treatment (Stocking-rate equivalent)	31 July	28 Aug.	4 Oct.	19 Oct.	30 Jan. 1957
0	42.3	42.4	42.9	38.5	21.7
4	42.5	42.4	44.0	36.3	—
8	41.9	41.9	41.6	34.5	—
12	42.1	42.4	41.2	33.7	—
16	41.5	40.4	41.8	33.6	—
20	43.4	40.6	39.7	32.0	20.5
S.E. ±	1.0	0.9	1.0	0.7	0.8
Sig. diff. at 5% level	N.S.	N.S.	N.S.	2.2	2.3

Soil Volume Weight

On 2 September 1956 there was a significant trend towards increases in volume weight with increases in treading rate, at all depths. At the 6.0–7.5 cm depth, a limited number of samples failed to show significant differences between treatments (Table 6).

TABLE 6. SOIL VOLUME WEIGHTS (2.9.56)

Treatment (Stocking-rate equivalent)	Depths			
	0.0–1.5 cm	1.5–3.0 cm	3.0–4.5 cm	4.5–6.0 cm
0	1.08	1.17	1.21	1.20
4	1.08	1.18	1.20	1.26
8	1.07	1.22	1.26	1.25
12	1.12	1.22	1.27	1.28
16	1.13	1.27	1.29	1.27
20	1.16	1.26	1.28	1.28
S.E. ±	0.019	0.020	0.018	0.024
Sig. diff. at 5% level	0.06	0.06	0.05	0.07

Ryegrass Seed Heads

There was a significantly greater number of seed heads on the heavily trodden plots than on the lightly trodden plots (Table 7).

TABLE 7. RYEGRASS SEED-HEAD COUNTS

Treatment (Stocking-rate equivalent)	No. Heads/sq.yd
0	345
4	324
8	395
12	484
16	496
20	537
S.E. ±	38
Sig. diff. at 5% level	111

Observations

Tiller and node vigour were reduced by treading, and although some died, the effect was masked by new growth. Round, tufted grass plants tended to become elliptical under treading, lying lengthwise across the plot, while growth form changed from erect to prostrate. *Rumex* spp., *Crepis capillaris* (L.) Wallr., *Taraxacum officinale* Weber, and *Veronica serpyllifolia* L. were moderately tolerant of treading, while *Cerastium glomeratum* Thuill. disappeared after the second treading.

During the first treading, surface-soil aggregates were destroyed progressively, the most obvious change occurring at the 10–12 sheep level; at heavier intensities the surface soil was puddled. The second treading produced this effect at the 8–10 sheep level. In October, the puddled surface soils cracked as they dried, while at the 1.5–4.5 cm depth a bluish colour (gleying) was observed under treatments heavier than the 4-sheep equivalent, notably under the 20-sheep equivalent treatment. Earthworms were seen on the surface of untrodden plots one day after mowing, whereas in 20-sheep plots they were first seen five to eight days later.

DISCUSSION

Generally speaking, the technique was practicable but there were some problems. The walking of driven sheep appeared to differ from that of set-stocked sheep. At the start of each treatment they tended to run and cause appreciable damage through slipping and sliding, whereas when they were tired, their shorter strides, implying more numerous hoofprints, were also damaging to the pasture. By constantly changing the starting point these effects were spread as evenly as possible over all the trodden plots. A man preceded the sheep and an another followed; their joint treading effect has been ignored, as it was probably small. Slow sheep could be forced along with the mob but it was difficult to keep the fast sheep back. Hence the middle of each strip was probably trodden 10–20% in excess of the proposed treatments. As the treatments were completed within 4 hours on each occasion, natural changes in soil condition during treatment were likely to be small. The quality of the walking was unknown but it appeared that the treatments were akin to a special kind of mob stocking. In a recent study, Cresswell (1957) estimated that mature Romney sheep walk about 1.1 miles per day on flat pasture similar to that used here, so that the 4-sheep equivalent used here might more properly have been called 6-sheep equivalent.

Little dung and urine was voided on to the plots and the initial fertility appeared to be adequate for the experiment, although all plots appeared to lose vigour before the last harvest.

There were probably defoliation effects, as O'Connor (in press) reported that long herbage reduced treading damage and in this experiment the herbage was mown short when trodden. Further, the action of the mower roller may have aggravated the damage. Perhaps the low-growing plants of heavily trodden plots might have escaped harvest, but yield and botanical composition data secured (not shown) for quadrats cut to $\frac{1}{4}$ in. height showed differences of the same order as for the 1 in. height. As all treaments were applied together, it is possible that heavily trodden plots, in which botanical composition and plant density were altered, were defoliated and trodden more often than was consistent with sustained pasture vigour. Thus the data produced by the first treading are particularly important.

It appeared that, in this experiment, the technique permitted study of treading under mowing rather than under grazing conditions and that significant differences due to treatment of the order of 10–15% could be detected.

Cumulative effects might better be estimated by subjecting differently trodden plots to different growth periods, depending on plant density, production, etc.

Initially, the structure of the soil was quite good, but, as it was plastic at treading, it was modified and plants were affected indirectly as well as directly. The recovery and reappearance of damaged and buried tillers were remarkably rapid. Despite a reduction immediately following treading, the ryegrass tiller density was near normal at each subsequent sampling. A pertinent explanation is offered by Brougham (1956) who stated that, in similar pasture, rate of plant growth per unit area increases until all light energy is intercepted. Growth and development of grass plants may thus have been nearly constant in untrodden and lightly trodden plots, whereas in the absence of competition, the open swards of heavily trodden plots grew and developed more rapidly. White clover did not, however, respond in this way, owing to winter dormancy.

No doubt the lower herbage yields from heavily trodden plots was due in part to low pasture density immediately after treading, and to the inability of immature tillers to grow as much herbage as mature tillers. However, as white clover stems were peculiarly susceptible to treading and as soil conditions were not conducive to activity by *Rhizobium* spp. (Butler and Bathurst 1956) it is possible that the available soil nitrogen in these plots was supplied by decomposition of plant residues and mineralisation of other available soil nitrogen. Perhaps, in the absence of dung and urine, the supply was inadequate, and the reduced vigour of the persistent mature ryegrass tillers in heavily trodden plots appeared to support this view, in part.

However, soil compaction occurred, a soil pan being formed at 4–6 cm depth, which, together with puddling of the surface soil, impeded drainage and gaseous diffusion (Domby and Kohnke 1956). Chlorosis of the plants growing in such soil suggested a deficiency, possibly of oxygen, as indicated by a bluish colour (gleying) at 1.5–4.5 cm depth. It is unlikely that the soil was sufficiently dense to limit root growth (Veihmeyer and Hendrickson 1948). Earthworm activity, drying of the soil, and plant growth, appeared to counteract in part the effects of treading. The different soil conditions may have caused the pattern of ryegrass seed-head production, e.g. soil moisture higher in untrodden plots in drought, but in lightly trodden plots seasonal clover growth could have restricted ryegrass development by shading and competition for available soil nitrogen (Willoughby 1954).

From the regression analysis it was seen that there were no critical intensities of treading, as all increases in rate produced proportional increases in damage.

It is notable that the first treading produced most of the measured effect, and that changes in botanical composition were more persistent than were reductions in yield. Indeed, total herbage yield data (not shown) secured 22 days after the final experimental cut did not show any significant differences due to treatment, but white clover was still affected.

Although the experimental technique allowed study of only one kind of mob stocking, the importance of treading on pastures, at all intensities, has been clearly demonstrated.

ACKNOWLEDGEMENTS

The author acknowledges the assistance of Mr. E. O. C. Hyde, who suggested the problem and the technique, and Miss D. P. Owen. Mr. M. Gradwell, Soil Bureau, and Mr. A. C. Glenday, Applied Mathematics Laboratory, also gave willing co-operation.

REFERENCES

BATES, G. H. 1930: The Effect of Consolidation upon the Botanical Composition of Poor Grassland. *J. Minist. Agric. 37:* 583-9.

——— 1935: The Vegetation of Footpaths, Sidewalks, Cart-tracks and Gateways. *J. Ecol. 23:* 470-87.

——— 1951: Track Making by Man and Domestic Animals. *J. Anim. Ecol. 20:* 21-8.

BROUGHAM, R. W. 1956: Effect of Intensity of Defoliation on Regrowth of Pasture. *Aust. J. agric. Res. 7:* 377-87.

BUTLER, G. W.; BATHURST, N. O. 1956: The Underground Transference of Nitrogen from Clover to Associated Grass. *Proc. 7th int. Grassl. Congr.* pp. 168-78.

CHANDLER, R. F. 1940: The Influence of Grazing upon Certain Soil and Climatic Conditions in Farm Woodlands. *J. Amer. Soc. Agron. 32:* 216-30.

CRESSWELL, E. 1957: A New Technique for Ranging Behaviour Studies. *Brit. J. Anim. Behaviour 5:* 119-20.

CROCKER, R. L. 1953: Soil and Ecological Problems Related to Chronic Overgrazing. *Proc. 6th int. Grassl. Congr. 1:* 770-5.

DAVIES, W. 1938: Vegetation of Grass Verges and Other Excessively Trodden Habitats. *J. Ecol. 26:* 38-49.

DOMBY, C. W.; KOHNKE, H. 1956: The Influence of Soil Crusts on Gaseous Diffusion. *Proc. Soil Sci. Soc. Amer. 20:* 1-5.

KLECKA, A. 1937: Influence of Treading on Associations of Grass Growth. *Herb. Abstr. 8:* 822.

KUDELIN, B. P. 1952: The Effect of Grazing on the Stability of Soil Structure, the Root System and the Yield of Perennial Herbage. *Augsne. un Raza 2:* 119-46.

LEVY, E. B. 1926: The Grasslands of New Zealand. Series II. The Mid-West North Island Hill Country. *N.Z. J. Agric. 32:* 291-310.

LIETH, H. 1954: Die Porenvolumina der Grunlandboden und ihne Beziehungen zur Bewirtshaftung und zum Pflanzenbestand. *Z. Acker-u. PflBau. 98:* 453-60.

MCCULLOCH, W. J.; CONNELL, R. P. 1934: Diversification as a Means of Making Dairying Safer and More Profitable. I. Potentialities in Fat Lamb Production on Portions of Some Dairy Farms. *N.Z. J. Agric. 48:* 277-84.

MITCHELL, K. J.: GLENDAY, A. C. 1958: The Tiller Population of Pastures. *N.Z. J. agric. Res. 1:*

O'CONNOR, K. F. 1956: Influences of Wheel and Foot Treading on Grasslands. *Proc. N.Z. Soil Sci. Soc. 2:* 35-7.

STEINBRENNER, E. C. 1951: Effect of Grazing on Floristic Composition and Soil Properties of Farm Woodlands in South Wisconsin. *J. For. 49:* 906-10.

VEIHMEYER, F. J.; HENDRICKSON, A. H. 1948: Soil Density and Root Penetration. *Soil Sci. 65:* 487-93.

WILLOUGHBY, W. M. 1954: Some Factors Affecting Grass-Clover Relationships. *Aust. J. agric. Res. 5:* 157-80.

EFFECTS OF FLOWERING ON THE ROOT SYSTEM AND SUMMER SURVIVAL OF RYEGRASS

By KATHLEEN SOPER*, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 10 December 1957*)

Summary

Tillers from a clone of short-rotation ryegrass, each with two or three roots, were planted out on three dates, 23 October, 15 November, and 14 December 1956. In half the plants all new roots were cut off as they appeared.

The number of flower heads formed decreased with later dates of planting. In the plants whose root systems were restricted to two or three roots, this decrease was associated with an increase in the period between dying back of the flower heads and death of the plant: in the earlier- and more profusely-flowering plants the vegetative tillers died when the flowering tillers died back in the autumn, while in the later- and less profusely-flowering plants, the non-flowering tillers remained alive into the winter or spring.

By the time the treated plants had died, the roots had lost most of the cortex and root hairs from all except the finest branches. In the intact plants, sloughing of the cortex did not occur at flowering, but was evident subsequently in some of the older roots.

It is concluded that two important factors determining the length of life of the roots of the parent flowering stem are the proportion of flowering to non-flowering tillers and the formation of roots by vegetative tillers. This is discussed in relation to annual and perennial habit in the ryegrasses.

INTRODUCTION

In ryegrass there is a trend in decreasing persistency from the perennials such as Kentish indigenous and New Zealand certified perennial through intermediates such as New Zealand certified short-rotation and New Zealand certified Italian to true annuals such as Western Wolths. This trend is accompanied by an increasing tendency for tillers to flower. In perennials the tillers need exposure to winter conditions (short day and/or low temperature, Cooper 1950) before they will flower. Therefore many remain vegetative and the plant continues in a flourishing state. Western Wolths, however, has no such requirement and nearly all the tillers flower and the plant normally dies.

When the seed heads die back, the persistence of the plant will depend on the subsidiary vegetative tillers which can survive only by either forming and establishing roots, or by using the roots of the parent tillers. In the case of an annual such as Western Wolths, if the season is dry, and grazing is hard, causing soil surface drought, the few remaining non-flowering tillers cannot establish roots; that is, the first alternative is not possible. In the case of a perennial, many of the vegetative tillers are formed before conditions become dry, and can establish roots and are thus better able to withstand drought.

---

* Now Mrs. C. S. W. Reid.

N.Z. J. agric. Res. 3: 329–340.

According to Skripcinskii (1955), it has been generally recognised that in perennial grasses the fertile stems, together with the roots that belong to them, die off completely after seed ripening. Cepikova (Weinmann 1948, p.116) found that in *Festuca pratensis* and *Phleum pratense* death of the old root system, as indicated by the cessation of catalase activity, coincided with the stage of most active growth of the plants, when inflorescences were emerging. Skripcinskii however, quotes Smevlov's conclusion that in *P. pratense* the roots of fertile stems continue to function after seed ripening into the following vegetative season. From his own experiments with cultivated and wild perennial rye and an *Agropyron* species, Skripcinskii concluded that roots of reproductive shoots do not die off after these shoots have fruited and dried but function until the start of winter, providing for the formation and subsequent growth of the sprouting shoots.

No information was available on the length of life of the roots of flowering stems in the ryegrasses, and this experiment was designed as a preliminary investigation to help elucidate the question.

METHODS

Groups of single tillers from a clone of New Zealand certified short-rotation ryegrass were planted out on three dates, 14 October, 5 November, and 3 December 1956. It was expected that this would give a range of flowering to non-flowering plants. After about ten days in moist sand, 10 tillers, each with 2 or 3 leaves, were selected from each group for replanting into pots of soil. All but 2 or 3 of their roots were cut away. Black braided nylon thread was tied loosely around each of these remaining roots to aid future identification. In half the plants all new roots were cut off as they were formed. To facilitate this the top inch of soil in each pot was replaced by sand (Fig. 1) which was kept dry by watering from below. All plants, except two in the November planting, a treated and a control, were shaded at the base to restrict tillering and root formation. This was done by surrounding the plant with a wire frame covered by two layers of scrim, the height of the scrim being increased as the plant grew (Figs. 2–6). Nitrogen was applied as urea in quantities just sufficient to keep the control plants green.

RESULTS

Flowering Behaviour

Data on flowering behaviour are given in Table 1. Observations from a limited number of treated plants only are included. In some cases it proved impossible to cut off all the new roots without damage to the original two or three, so that some plants eventually had more than two or three roots. A few plants were damaged and died before the end of the experiment. No data from these plants (except for TR6 from the December planting) are presented.

No consistent differences were observed between those plants that had two roots and those that had three. Those that developed more than two or three roots were generally intermediate in appearance between successful treatments and the controls.

TABLE 1. DATA ON FLOWERING OF INDIVIDUAL PLANTS

Date of Planting	Plant	Date of Harvesting	Duration of Experiment (days)	Total Tillers[1]	Flowerheads		Vegetative Tillers[1]		Period over which Flowerheads Emerged	Date of Death of Last Flowerhead	Number of Days Between Death of Last Flowerhead and Death of Plant
					Total	Dead at Harvest	Total	Dead at Harvest			
23.10.56	TR1	18.3.57	146	47	10	10	37	37	28.11.56 – 24.1.57	18.3.57	0
	C1	”	”		32	11			26.11.56 – 26.2.57	—	*
	C2	”	”		35	12			28.11.56 – 26.2.57	—	*
	C3	”	”	243	37	7	195	0	3.12.56 – 4.3.57	—	*
	C4	”	”		48	8			7.12.56 – 4.3.57	—	*
	C5	”	”		72	26			26.11.56 – 26.2.57	—	*
15.11.56	TR2	23.4.57	159	37	8	8	29	29	14.12.56 – 28.1.57	23.4.57	0
	TR3	13.5.57	179	21	2	2	19	19	28.12.56 – 21.1.57	26.2.57	76
	TR4[2]	26.9.57	315	75	8	8	67	64[1]	21.12.56 – 7.3.57	6.5.57	143+
	C6	23.4.57	159	185	24	17	161	0	24.12.56 – 1.4.57	—	*
	C7	13.5.57	179		1	1			(14.1.57)	18.3.57	*
	C8	”	”		22	14			24.12.56 – 25.3.57	—	*
	C9	”	”		36	29			21.12.56 – 25.3.57	—	*
	C10[3]	”	”		0	0			—	—	*
14.12.56	TR5	26.9.57	286	38	4	4	34	21[4]	21.1.57 – 12.2.57	4.6.57	114+
	TR6				0	0			—	—	?
	C11	13.5.57	120		9	8			31.1.57 11.3.57	—	(damaged)
	C12	16.7.57	214	327	30	30	297	26	14.1.57 – 15.4.57	15.7.57	*
	C13	”	”	309	1	1	308	2	(15.2.57)	24.6.57	*
	C14	13.9.57	273	302	16	16	286	47	24.1.57 – 25.3.57	5.8.57	*
	C15	”	”		2	2			8.1.57 – 31.1.57	9.4.57	*

* Vigorously growing when harvested.

1. Tillering data were obtained for only some plants.
2. and 3. Unshaded treated and unshaded control plant respectively.
4. Although plants were practically dead, remaining tillers had some green leaves.

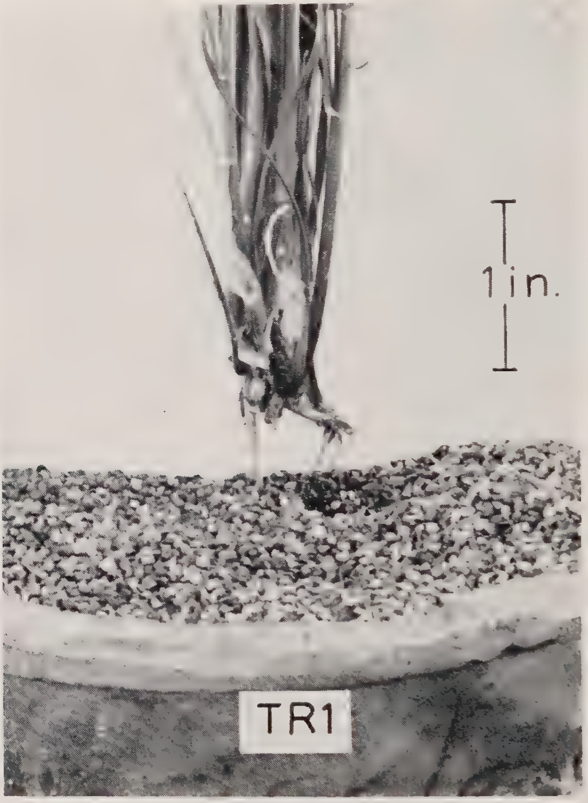


Fig. 1.—Base of the shoot of a treated plant (the whole plant is shown in Fig. 2), with the tops of the two uncut roots exposed. A wire support is shown on the left hand side of the plant.

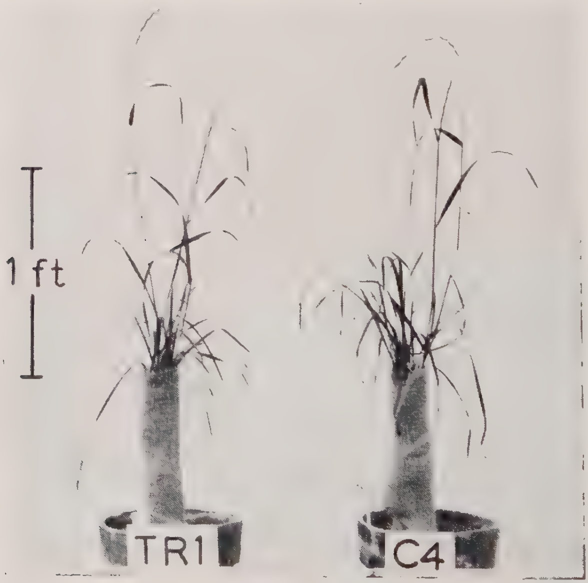


Fig. 2.—Plants from the October planting, on 14.12.56, i.e. 52 days after planting. (TR = treated, C = control).

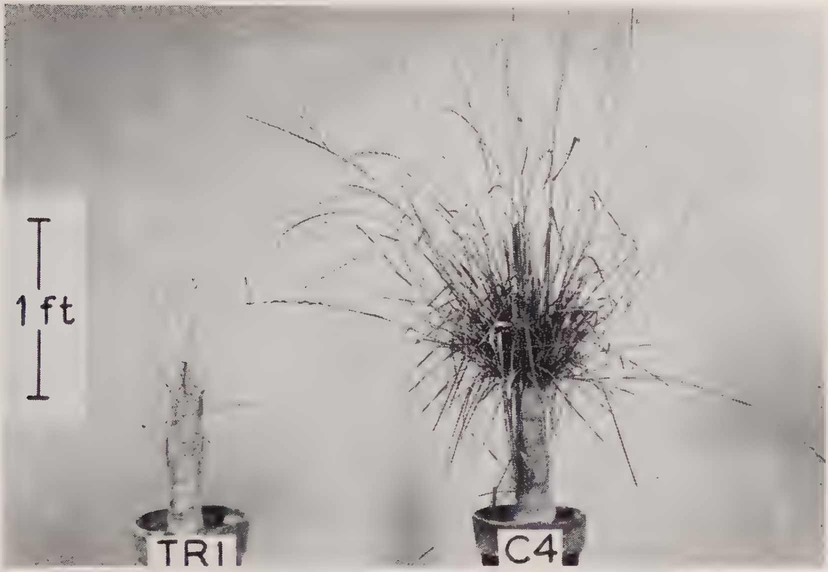


Fig. 3.—Plants from the October planting. 14.3.57.

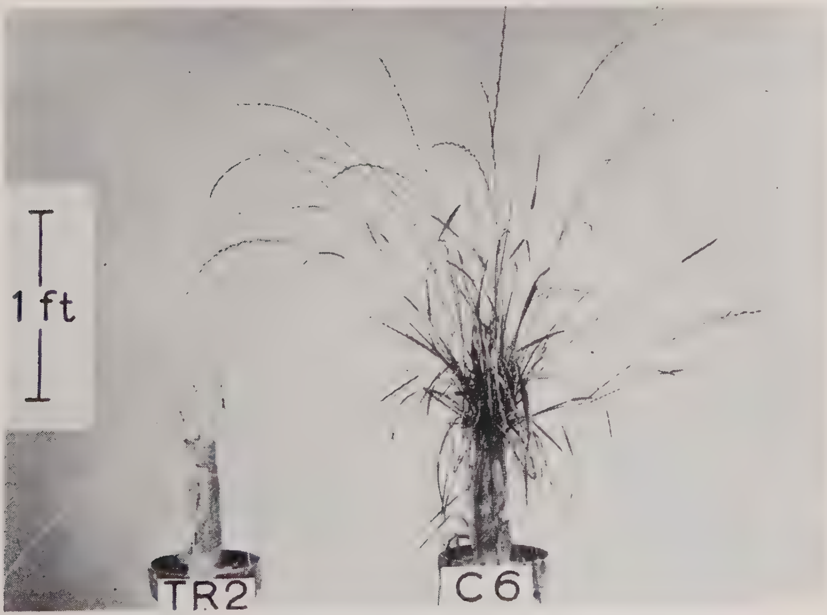


Fig. 4.—Plants from the November planting, 14.3.57.

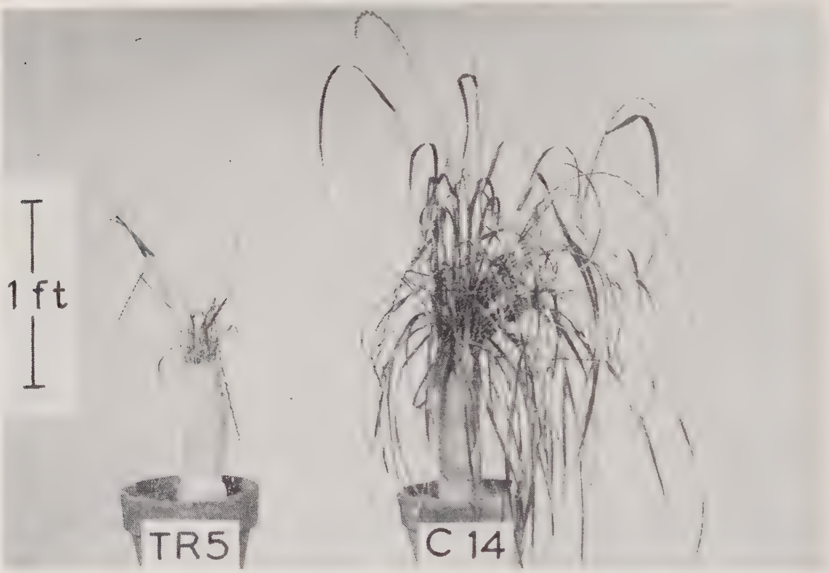


Fig. 5. Plants from the December planting, 14.3.57.

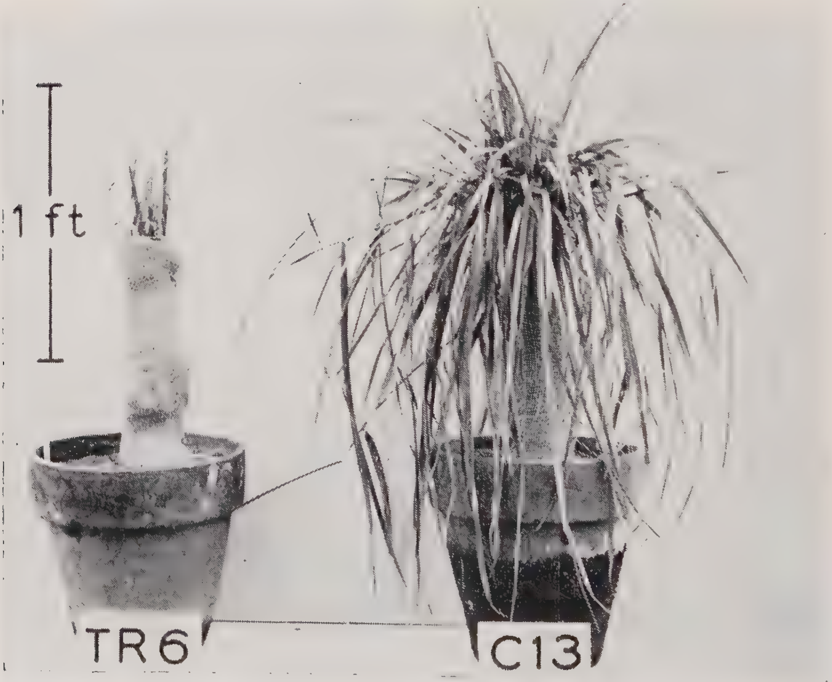


Fig. 6.—Other plants from the December group, 14.3.57.

Some 52 days after the October planting, there was little difference between the treated and control plants (Fig. 2). However, three months later (March) the treated plant was almost dead while the control plants were flourishing (Fig. 3), although about 25% of the flower heads on the latter had begun to die back (Table 1). At this time (March) treated plants from the November and December plantings were also clearly affected (Figs. 4, 5, and 6), but lived on for a few weeks more (with the exception of TR4, see Table 1) in the first case and for a few months more in the second.

It will be observed from these figures and from Table 1, that in the treated plants the number of tillers supported during the experiment was much smaller than in the control plants. The number of flower heads was less, and the flowering period shorter, except in a few cases where the later-planted controls had few or no flower heads. Flowering tillers were less in the November group than in the October group and less still, on an average, in the December group. There was increasing variation in the number of flower heads in the last two groups.

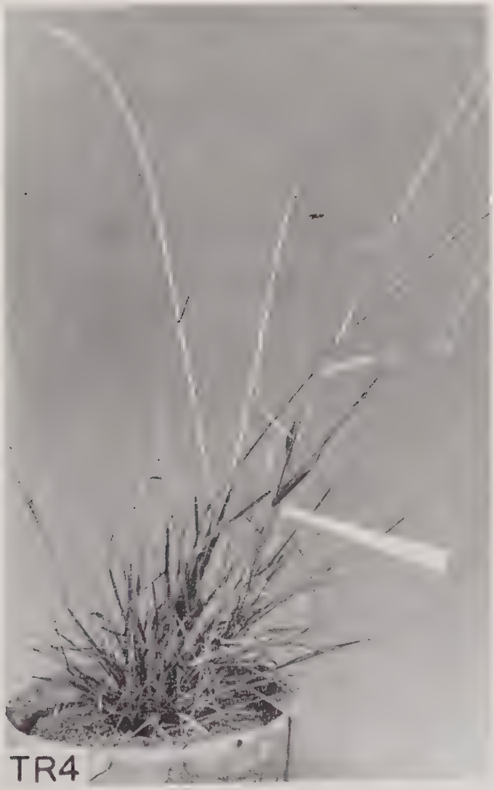


Fig. 7.—The unnshaded treated plant from the November group, on 14.3.57, showing a green tiller borne part-way up an apparently dead flowering stem (arrow).

In the treated plant from the November group shown in Fig. 4 (TR2), as in the treated plant from the October group, the vegetative tillers died as the last of the flower heads died back. In another treated plant from the November group (TR3, Table 1) however, as in that shown in Fig. 5 for the December group (TR5, Table 1), the vegetative tillers remained alive for some time after the dying back of the flowering stems.

This was also the case in the one unshaded treated plant, from the November group (TR4, Table 1). Fig. 7, taken at the same time as Figs. 3–6, shows this plant. It is of interest that a tiller developing from a node part-way up a culm remained green for two to three months after the seed head died back, which indicates that water and nutrients were still being transported between that point and the stem base. This was also frequently seen in base-shaded control plants from all groups.

The Roots

Examination of the roots was limited by the difficulty of extricating the fragile roots of the treated plants from the soil. Generally, in the treated plants, by the time the tops had died, the roots had lost the cortex and root hairs from all except the finest branches. In contrast, all the roots of the control plants from the October group at harvest (146 days after planting, and with about 25% of the flower heads apparently dead) were relatively thick, with cortex and densely spaced root hairs present throughout. Of the November group at harvest, in those control plants that had a number of flower heads, (159-179 days after planting and with about 70% of the flower heads apparently dead) there was evidence of cortex sloughing in up to half of the nodal roots. In the December control group at harvest (more than 210 days after planting and after all the flower heads had died back) 45-70% of the roots had the cortex sloughing from their older parts, irrespective of the number of flower heads borne by the plant.

DISCUSSION

Root Pruning and Shoot Growth

It is not unexpected that the treatment used in this experiment, that of restricting the root system to two or three nodal roots, should markedly reduce top growth. Severe root-cutting will restrict the uptake of water and nutrients by the plant. Further, it will reduce the quantity of the unidentified essential leaf growth factor produced by the roots (de Ropp 1945, 1946a, 1946b, 1947; see also Gregory 1956). Root pruning similarly reduced top growth in the experiments of Robertson (1933), Nedrow (1937) and Jacques and Edmond (1952). Jacques and Edmond found also, however, an *increase* in yield for the first few weeks in root-pruned plants, and faster root initiation. A root meristem does not synthesise its own organic nitrogenous materials but depends on a supply from basal regions (Heyes and Brown 1956). It appears that cutting off the root tips results in some of these materials becoming available for redirection to other growing regions, that is, to the stem apex and leaf primordia or to new roots. Continued cutting may deplete these supplies, both by loss in excised tissue and by bleeding from wounds, and this may be a further factor in the subsequent reduction in top growth.

In this experiment, the magnitude of the effect of the treatment on shoot growth indicates (1) that the ability of the remaining roots to compensate for the removal of new roots, whether by drawing on functional reserves, or by hypertrophy, is limited, and (2) that a balance between root and shoot growth is necessary for the continuance of vigorous plant growth. This is borne out by the fact that those plants in which more than the desired number of roots had grown were intermediate in shoot growth between the successfully treated and the control plants. It is clear, therefore, that the treated plants were under stress, and that the severity of this stress increased as the shoots grew. Superimposed upon this were the demands of flowering and seed production.

Effects of Flowering on the Root System and Survival of the Plant

After flowering, the seed heads die back and, to persist, a plant must depend on the tillers that have remained vegetative. The latter must either have already established roots or be able to continue using those of the parent until conditions favour the development of new roots.

In this experiment, the number of flower heads formed by each plant decreased with later dates of planting. In those plants whose root systems were restricted to two or three nodal roots, this decrease was associated with an increase in the period between dying back of the flower heads and death of the plant: in the earlier-planted, more profusely flowering plants the vegetative tillers died when the flowering tillers died back in the autumn, while in the later-planted, less profusely flowering plants, the non-flowering tillers remained alive into the winter or spring (Table 1).

In the treated plants, by the time the tops had died, the cortex had sloughed from the older parts of the roots. The state of the cortex during flowering was not observed. Presumably, the stress on the roots caused by flowering was greater in the more profusely flowering plants, with the result that eventually the vegetative tillers could not obtain through the parent's roots water and nutrients sufficient for their needs. The explanation of this may be either (1) that the absorbing region remaining after the cortex had sloughed off the greater part of the roots was inadequate to supply the needs of the vegetative tillers, or (2) that growth of the distal parts of the roots was inhibited by a substance transported from the dying flower heads. Another explanation for the death of the plant could be (3) that the storage regions were inadequate to cope with the reserves restored to the roots during maturation.

There is some evidence to support each of these possibilities. Although the region of greatest absorption and translocation of nutrients and water is the root tip (Goodewaagen 1955, pers. comm. to Troughton 1957) or the region 30–50 mm back from the root tip (Wiebe and Kramer 1954), the region above that also absorbs, though to a lesser extent (*loc. cit.*). Wiebe and Kramer used roots 5–6 days old. Goodewaagen used the "bleeding" technique on roots where the cortex was sloughing off the older parts, and in these the absorptive region above the root tip was limited to those parts upon which the cortex remained. Jacques (1956), Jacques and Schwass (1956), and Schwass and Jacques (1956) have concluded from their experiments that roots that have lost the cortex from their older parts are less efficient as absorbing organs than intact roots, and that only the distal parts of such roots function in absorbing nutrients and water.

Moiseeva (1956) found that root growth in maize was impeded by phytohormones from oat stems at flowering. It may be that root growth in a plant is impeded by phytohormones from its own dying flower heads. It is known that carbohydrates, nitrogen, potassium, and phosphorus are translocated from tops to roots after flowering (Weinmann 1940, 1948). It is not improbable that phytohormones could also be transported from the dying flowering stems to the roots. The magnitude of the effect would appear to depend on the number of tillers that formed flower heads, since plants with proportionally few flowering stems did not die immediately after dying back of the flower heads. Whether or not phytohormones from dying flower heads do in fact impede root growth is a point worthy of further investigation.

With regard to the third possibility, evidence for the restoration of reserves to roots during maturation of the seeds has been obtained by Aldous (1930), McIlvanie (1942), Dilz (1956) and others. In the treated plants the storage regions would be limited, and the situation could be envisaged where a general choking up of the system could occur.

In the control plants, the duration of life of the roots of fertile stems was not determined. They appeared to be still alive at harvesting. The time of death might be determined by testing the absorption by these roots of radio-active isotopes or by histochemical methods. At harvest, the cortex was almost intact in plants from the October planting, while those from the November planting showed sloughing in some of the roots. In plants from the December planting, which were harvested after all the flower heads had died back, the cortex was sloughing in half or more of the roots, irrespective of the number of flower heads borne by the plant. Thus it would appear that sloughing of the cortex in the intact plants, although possibly correlated with age of the plant, season, or dying back of the flower heads, is not correlated with flowering. Since a reduction in quantity of reserves in the roots has been reported as taking place before or at the time of flowering (Weinmann 1952; Dilz 1956; Nielsen and Lysgaard 1956), it might have been expected that sloughing of the cortex also would have occurred at that time (Jacques 1956; Soper 1957). That this was not the case may mean that some reserves still remained in the cortex of the roots of the fertile stems after shooting, possibly because the proportion of flowering to non-flowering tillers was never greater than 20%.

Clearly, much remains to be found out about the correlation between sloughing of the cortex and quantitative changes in reserves in the roots during flowering, seed ripening, and dying back of the flower heads.

Factors Affecting Persistence of Roots. Whatever the mechanisms involved, it is clear from this experiment that two important factors determining the continued functioning of parent roots after seed-ripening into the following vegetative phase are (1) the proportion of flowering to non-flowering tillers; the greater the number of flower heads the shorter is the life of the roots after the flower heads die back, and (2) the formation of roots by vegetative tillers: such roots relieve stress on the parent roots during and after flowering.

In annuals, where most of the tillers bolt to seed, a situation similar to that in the more profusely flowering treated plants might arise, especially in cases where the few remaining vegetative tillers cannot establish roots. Jacques and Schwass (1956) reported for Italian ryegrass a decline in root and tiller numbers during January and until the end of March. They suggested that this might be due to the relatively short effective life of the roots, together with a cessation in the initiation of new roots.

Requirements for Summer Survival of Ryegrass

From the viewpoint of persistency over a dry summer, a requirement for ryegrass appears to be a short, early flowering period during which tillers formed before a certain date (determined by day length and temperature) flower, while those formed after that date remain vegetative and develop a substantial root system before the dry season sets in. Perennial ryegrass has this short, early flowering period, but has the disadvantages of less rapid establishment, lower winter production and lower palatability than Italian and short-rotation ryegrass. Short -rotation ryegrass is intermediate in persistency between Italian and perennial, less productive in the autumn than perennial, and also less able to tolerate hard grazing. Its relative persistency is largely influenced by grazing management; under a lenient system of grazing combined with high soil fertility it approximates to a perennial, but under continuous hard grazing it may be little better than an annual (Corkill 1950). As well therefore, as a ryegrass with the flowering behaviour outlined above, a type of pasture management is desirable that reduces grazing to a minimum during drier periods when hard grazing would (a) cause partial or complete stoppage of growth in roots that have already established themselves (Crider 1955), and (b) deplete the vegetative cover so that the soil surface dries out and any new roots cannot establish themselves.

ACKNOWLEDGEMENTS

The writer is indebted to Mr. E. O. C. Hyde who suggested and discussed this work, to Miss D. P. Owen for technical assistance, and to Mr. H. Drake for the photography.

REFERENCES

ALDOUS, A. E. 1930: *J. Amer. Soc. Agron. 22:* 385-92.
COOPER, J. P. 1950: *J. Brit. Grassl. Soc. 5(2):* 105-112.
CORKILL, L. 1950: *N.Z. J. Sci Tech. A32(3):* 35.
CRIDER, F. J. 1955: *U.S. Dep. Agric. Tech. Bull. 1102.*
DILZ, K. 1956: *Landbouwk. Tijdschr. 68:* 12, 947-51.
GREGORY, F. G. 1956: In MILTHORPE, F. L. (Ed.) 1956: "The Growth of Leaves". *Proc. 3rd Easter Sch. agric. Sci. Univ. Nottingham, 1956:* pp. 3-17. Butterworths Scientific Publications, London.

HEYES, J. R.; BROWN, R. 1956: In MILTHORPE, F. L. (Ed.) 1956: "The Growth of Leaves". *Proc. 3rd Easter Sch. agric. Sci., Univ. Nottingham, 1956.* pp. 31-49. Butterworths Scientific Publications, London.

JACQUES, W. A. 1956: *N.Z. J. Sci. Tech. A38:* 160-5.

JACQUES, W. A.; EDMOND, D. B. 1952: *Ibid. A34:* 231-48.

JACQUES, W. A.; SCHWASS, R. H. 1956: *Ibid. A37:* 570-83.

MCILVANIE, S. K. 1942: *Plant Physiol. 17:* 540-57.

MOISEEVA, M. N. 1956: *Bot. Zh. SSSR 41(4):* 560-8. (*Herb. Abstr. 27:* 285).

NEDROW, W. W. 1937: *Ecology 18:* 26-52.

NIELSEN, Hj. M.; LYSGAARD, C. P. 1956: *K. Vetllojsk. Aarsskr.* 77-107. (*Biol. Abstr. 31:* 18691)

ROBERTSON, J. H. 1933: *Plant Physiol. 8:* 425-47.

DE ROPP, R. S. 1954: *Ann. Bot. n.s. 9:* 369-81.

————1946a: *Ibid. 10:* 31-40.

————1946b: *Ibid. 10:* 353-9.

————1947: *Ibid. 11:* 439-47.

SCHWASS, R. H.; JACQUES, W. A. 1956: *N.Z. J. Sci. Tech. A38:* 109-19.

SKRIPCINSKII, A. V. 1955: *Doklady Akad. Nauk SSSR 101(2):* 371-3. (*Herb. Abstr. 25:* 888:*Biol. Abstr. 13:* 15238.)

SOPER, KATHLEEN, 1957: *N.Z. J. Sci. Tech. A38:* 783-92.

TROUGHTON, A. 1957: "The Underground Organs of Herbage Grasses". Commonwealth Agricultural Bureaux, England. 163 pp.

WEINMANN, H. 1940: *Plant Physiol. 15:* 467-84.

————1948: *J. Brit. Grassl. Soc. 3(2):* 115-40.

————1952: *Proc. 6th int. Grassl. Congr.* pp. 655-60.

WIEBE, H. H.; KRAMER, P. J. 1954: *Plant Physiol. 29:* 342-8.

SEASONAL GROWTH RELATIONSHIPS IN ABERDEEN ANGUS × JERSEY CROSSBRED CATTLE

By M. A. MacDonald*, Ruakura Animal Research Station, Department of Agriculture, Hamilton

(*Received for publication, 17 December 1957*)

Summary

Twenty-one calves sired by purebred Aberdeen Angus bulls and out of commercial Jersey cows were brought to Ruakura Animal Research Station during the late winter of 1953. Bucket feeding commenced when calves were two days of age and each was fed according to Ruakura recommendations. Seven bull calves and 4 heifers were weaned at 8 weeks of age and the remaining 6 males and 4 heifers were weaned at 18 weeks. Males were castrated at ages ranging from 14 to 17 weeks. Growth rate was measured by live-weight gain and anatomical measurements until the animal reached 30 months of age. Data were analysed by Doolittle's method of least squares analysis, Brody's method for determining instantaneous growth constants, and analysis of variance. The following results were obtained:

1. Steers gained 133 pounds more than heifers during the 30-month experimental period. This difference was highly significant ($P = <.01$).
2. Age at weaning did not affect weight gain during either the first year or the entire experimental period.
3. Growth rate, measured by instantaneous growth constants for growth periods between ages of one and fifty-two weeks, decreased steadily with age for all groups.
4. Growth rates of all groups of crossbreds equalled that of pure-bred Aberdeen Angus cattle at Ruakura and exceeded that of range beef steers in Texas and feedlot steers fed a moderate T.D.N. ration in Missouri.
5. Anatomical measurements indicated that the period of pasture shortage during the second year did not suppress skeletal growth but did suppress live-weight gain and degree of fill.
6. Variation in live weight within seasons and within groups was sufficiently large to prevent expression of statistically significant growth relationships between seasons. Relative seasonal gains could not be predicted.

On the basis of this and other studies, crossbred calves for veal and light-weight beef production are recommended. If a pasture shortage necessitates a reduction in numbers carried beyond the veal stage, steers should be selected preferentially for retention.

Introduction

In New Zealand there is an annual slaughter of over a million predominantly Jersey calves within a few days of birth. It was proposed that by crossing poor butterfat-producing Jersey cows with bulls of a highly improved beef breed, it should be economical to produce a suitable veal or beef carcass. In an earlier study at this Station (Radford 1954) it was shown that crossbred calves of both sexes produced satisfactory

---

* Now: Physiologist, Animal Husbandry Division, Canada Experimental Farm, Lethbridge, Alberta.

N.Z. J. agric. Res. 3: 341–348.

veal carcasses following weaning at either 8 or 18 weeks of age. The possibility of carrying crossbred steers and heifers through to light carcass weights was also studied and reports of carcass quality and rates of gain were favourable (McMeekan 1956). An understanding of the significance of relationships between gains made on pasture by both sexes at different weaning weights and during different seasons is necessary when selecting animals to be vealed or carried through to beef carcass weights.

Few publications report the significance of relationships between gains made by cattle during different seasons or physiological periods. Knapp and Clark (1950) found heredity was most important in feedlot gains where environmental influences were minimised. Their results were based on phenotypic, genotypic, and environmental correlations between gains made during three sequential post-weaning periods of 84 days each. Koger and Knox (1951) reported that growth of cattle on New Mexican range was positively correlated with feedlot gains. They concluded that when environment is constant, relationships between gains made during different periods are positive. In a more recent paper, Kidwell (1954) found somewhat similar results. Urick *et al.* (1957) reported relationships between three successive seasonal growth periods in Hereford steers. They found highly correlated gains and concluded that selection in any one period should result in gain in the other periods.

This paper presents data from a study of growth and the relationship between gains made by early and conventionally weaned steers and heifers during successive grazing seasons.

EXPERIMENTAL PROCEDURE

Twenty-one Aberdeen Angus × Jersey calves comprising 13 bull calves and 8 heifers were purchased and brought to Ruakura during the late winter of 1953. Bucket feeding commenced when the calves were two days old and each animal was fed according to Ruakura recommendations for dairy calves (McMeekan 1954, 1956; Radford 1954). Seven bull calves and four heifers were weaned at 8 weeks and the others were weaned at 18 weeks of age. All calves were grazed together following a rotational grazing plan until 18 weeks of age when they were separated into monosexual groups while grazing the same pastoral area. Animals were weighed individually at weekly intervals until 28 weeks of age and at fortnightly intervals thereafter. Height at withers, heart girth, paunch girth, and hip-pin measurements were made on each animal at 4-week intervals commencing when calves were approximately 26 weeks of age. Bull calves were surgically castrated at ages ranging from 14 to 17 weeks.

Data were analysed using Doolittle's method of least squares analysis (Blair 1944) and Brody's method for determining instantaneous growth constants (Brody 1945; Williams 1956).

RESULTS AND DISCUSSION

Growth represented by average live weight of the four groups of experimental animals is presented in Fig. 1 and Table 1. It may be seen that growth rate was not depressed during the first winter but it

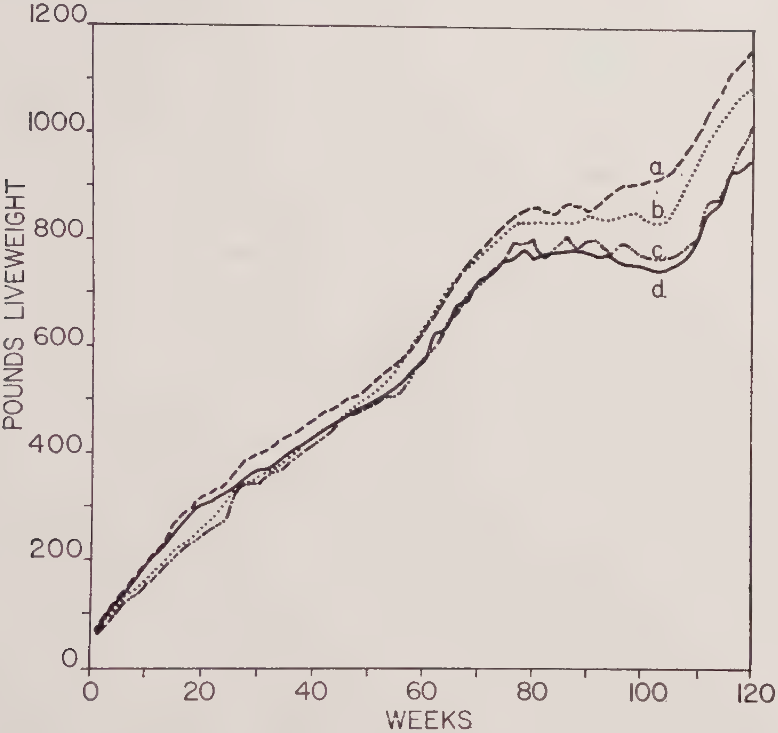


Fig. 1.—Growth of early and conventionally weaned Aberdeen Angus × Jersey steers and heifers.
(a) steers weaned at 18 weeks of age. (b) steers weaned at 8 weeks of age.
(c) heifers weaned at 8 weeks of age. (d) heifers weaned at 18 weeks of age.

TABLE 1. Average Daily Gains (lb.) of Crossbred Cattle by Seasons

Season	Year	Weaning Age			
		8 weeks		18 weeks	
		Heifers	Steers	Heifers	Steers
Birth—Spring	1953	1.49	1.38	1.65	1.81
Spring	1953	1.27	1.39	1.73	1.80
Summer	1953–54	1.32	1.40	0.96	1.19
Autumn	1954	1.17	1.14	1.05	0.88
Winter	1954	1.01	1.36	0.86	1.22
Spring	1954	1.97	1.93	2.04	2.14
Summer	1954–55	0.60	0.80	0.49	0.75
Autumn	1955	0.07	0.21	−0.15	0.55
Winter	1955	0.31	0.81	0.12	1.11
Spring—Final Wt.	1955	2.98	2.15	2.67	2.10
Average		1.15	1.23	1.07	1.31

was depressed seriously during the second winter. The cause of crossbred growth-rate depression was a pasture shortage resulting from dry summer weather coupled with higher maintenance requirements. Both steer groups were able to maintain weight during the period of feed shortage but both heifer groups lost weight. It is probable that the steers made greater use of pasture available than heifers, although possible differences in metabolism between steers and heifers cannot be ignored (MacDonald and Bogart 1955). Following the severe pasture shortage during the second year, all groups made compensatory gains.

Growth during various age periods between one and 52 weeks of age were analysed by Brody's method (Brody 1945; Williams 1956) and expressed in growth periods by means of an instantaneous growth constant, K, (Table 2). As expected, daily percentage growth rate decreased steadily in all groups as age and weight increased.

TABLE 2. INSTANTANEOUS GROWTH CONSTANTS (K) FOR GROWTH PERIODS BETWEEN AGES ONE AND FIFTY-TWO WEEKS

Analysis	Growth Period (weeks)	Weaning Age			
		8 weeks		18 weeks	
		Heifers	Steers	Heifers	Steers
1	1 – 8	.0154	.0153	.0172	.0180
2	1 – 18	.0110	.0104	.0118	.0124
3	1 – 26	.0096	.0089	.0090	.0096
4	1 – 52	.0059	.0057	.0055	.0058
5	8 – 18	.0079	.0071	.0079	.0084
6	8 – 26	.0041	.0036	.0033	.0036
7	8 – 52	.0043	.0041	.0037	.0039
8	18 – 26	.0065	.0057	.0032	.0039
9	18 – 52	.0043	.0043	.0031	.0037
10	26 – 52	.0024	.0025	.0021	.0022

Effect of Age at Weaning on Live-weight Gains

There was a slight but statistically insignificant temporary depression in growth rate of those animals weaned at 8 weeks compared with the conventionally weaned calves. During the period between 8 and 18 weeks of age the bucket-fed males gained considerably faster than weaned males, and females of the conventionally weaned group gained faster than those of the early-weaned group.

Between 18 and 26 weeks of age early-weaned groups averaged greater gains than conventionally weaned groups. This was probably due not only to compensatory gains by the early-weaned groups which had become conditioned to a pasture diet, but also to the dietary readjustments following weaning experienced by the conventionally weaned groups. At no time was there a statistically significant difference in growth rate between early- and conventionally weaned calves since differences within groups offset possible differences between groups (Table 3).

TABLE 3. Analysis of Variance: Total Gains of Early and Conventionally Weaned Aberdeen Angus × Jersey Steers and Heifers

Source of Variation	d.f.	S.S.	M.S.	F.
Total	20	193,622		
Diets	1	425	425	0.088
Sex	2	111,367	55,683.5	11.57**
Individuals	17	81,830	4,813.5	

**Significant at the 1% level

Effect of Sex on Live-weight Gains

Although age at weaning did not affect rate of growth, sex did. At 120 weeks of age, steers averaged 133 pounds heavier than heifers. Expressed as a percentage the difference in weight of steers over heifers was 14.3. This sex difference in total live-weight gain (Table 3) was highly significant (P =<.01) but it did not become apparent until after one year of age. Heifers weaned at 8 weeks of age grew at the highest instantaneous growth rate during the first 52 weeks but by 40 weeks of age the average live weights of both steer groups were higher than those of the heifer groups. Heifers also were less able to withstand the pasture shortage than steers during the second winter. Steers either maintained weight or grew slowly during this period but heifers lost weight. However, with the advent of spring pasture growth the heifer groups attained compensatory growth rates which exceeded those of the steer groups (Figure 1, Table 1).

Comparative Rates of Gain of Crossbred Cattle

Direct comparison with purebred Aberdeen Angus cattle was not possible since the latter were left to graze with their dams on similar pastures. However, these Aberdeen Angus cattle, grazing at similar stocking rates, grew at almost identical rates of gain as their crossbred counterparts. A comparison of growth rate of the Ruakura crossbreds with American cattle is illustrated in Fig. 2. Here it may be seen that each crossbred group exceeded the growth rate reported for Texas range-reared steers and heifers (Lush *et al.* 1930), and equalled the growth rate of Missouri steers fed a growing ration of moderate T.D.N. content. As a result of the pasture shortage during the late summer and winter of 1954–55, the Ruakura crossbreds were unable to maintain beyond 18 months of age a growth rate equivalent to that realised by well fed Minnesota steers (Lush *et al.* 1930).

Linear Size of Crossbred Cattle

Several formulae are available for estimating the weight of cattle from chest girth and the nutritive condition of cattle from height at

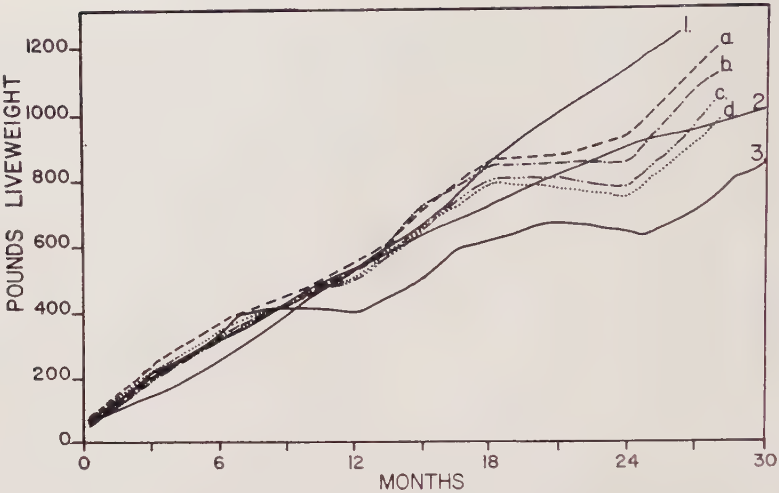


Fig. 2.—Growth of steers and heifers subjected to different systems of management.
1. Minnesota steers fed on a high plane of nutrition.
2. Missouri steers fed a growing ration of moderate T.D.N. intake.
3. Texas steers and heifers on pasture with supplemental feed available late in very severe winters.
Ruakura steers (a and b) and heifers (c and d) weaned on to pasture at 8 or 18 weeks; no supplemental feeds provided.

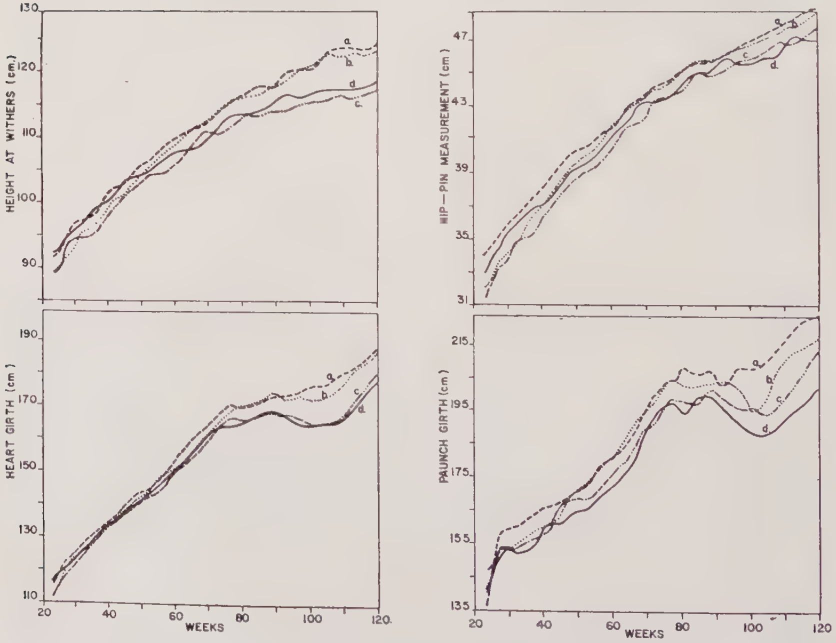


Fig. 3.—Effect of plane of nutrition on height at withers, hip-pin measurement, heart girth, and paunch girth. (a) steers weaned at 18 weeks of age. (b) steers weaned at 8 weeks of age. (c) heifers weaned at 8 weeks of age. (d) heifers weaned at 18 weeks of age.

withers. According to Brody (1945), heart girth (chest girth) comes nearest to meeting the theoretical expectation that weight (volume) varies with the cube of linear size. Height at withers is considered the best reference base for nutritive condition in cattle because height at withers, unlike heart girth, is relatively independent of environmental conditions (Brody 1945; Winchester and Ellis 1956; Winchester and Howe 1955). Paunch girth, measured immediately posterior to the last rib, is generally indicative of fill as well as live weight. The comparative effect of plane of nutrition expressed as pasture availability and sex on body measurements may be seen in Fig. 3. By one year of age, the two steer groups had greater height at withers than the heifer groups, and the difference gradually increased with age. The pasture shortage during 1954–55 had very little effect on height at withers. The hip-pin measurements which are also a measure of skeletal development were not affected by pasture shortage and were greater for steers than heifers after approximately one year of age. Sex dimorphism was not as great for hip-pin measurements as for measurements of height at withers.

Heart girth, the principal factor in estimates of body weight, followed the live-weight growth pattern of the crossbred cattle more closely than the other three measurements analysed (Figs. 1 and 3). This was particularly apparent during the period of pasture shortage. Both heifer groups lost weight and their heart girth diminished, but the steer group that continued to increase in live weight showed an increase in heart girth. During the period of compensatory weight gains, which were more rapid in the heifers than in steer groups, heart-girth increases were also comparatively more rapid in the heifer groups. Paunch girth was more variable than the other three measurements taken because of feed variables such as pasture intake, water intake, and rate of passage of feed. The effect of pasture availability during 1954–55 is reflected in the paunch girths of all groups of crossbreds during the age period of ninety to one hundred and fifteen weeks. It may be concluded that the period of pasture shortage was insufficiently severe to decrease skeletal growth rate measured by height at withers and hip-pin measurements but was severe enough to cause the heifer groups to lose weight, condition (heart girth), and fill (paunch girth). Undoubtedly more use could be made of paunch girths taken in conjunction with live weight when assessing livestock yields on pasture.

Seasonal and Total Weight Gain Interrelationships

It has been shown that it is feasible to wean bucket-fed crossbred calves at 8 weeks (Radford 1954), that quality of meat is satisfactory both for veal (idem) and lightweight beef (McMeekan 1956), and that live-weight gains on pasture approximate those of Aberdeen Angus cattle. Crossbred calves thus provide a potential source of supplemental income on dairy farms with no increase in the number of labour units required to manage such an enterprise. The greatest deterrent to raising crossbred calves, then, is during wintering periods when both dairy cattle and crossbred calves are forced to compete for available pasture. It would appear wise to veal poor-gaining calves according to Radford's (1954) recommendation and raise the better calves to beef carcass weights. Under such a management system it is necessary to establish criteria which enable those animals that will gain most quickly to be distinguished from those that will not. Up to slaughter weights for veal, efficiency of energy conversion is sufficiently high and pasture sufficiently plentiful to justify carrying all the crossbred calves (McMeekan 1956).

Live-weight gains of all 21 crossbred animals were analysed by seasons using Doolittle's method of least squares analysis (Blair 1944) in an attempt to determine if gains made on pasture during one season were sufficiently highly correlated with gains made during subsequent seasons that selection in any one period should result in greater gain per animal during subsequent periods. In no season was the variance ratio (F value) statistically significant. It must be concluded that variation in live-weight gains within seasons and within groups were sufficiently great to prevent the expression of statistically significant growth relationships between seasons. These results are in contrast with those reviewed previously whereby a positive significant correlation between growth during sequential periods was obtained (Kidwell 1954; Knapp and Clark 1950; Koger and Knox 1951; Urick *et al.* 1957). However, most of the studies cited were conducted under rigidly controlled environmental conditions or a rising plane of nutrition.

On the basis of this and earlier studies, crossbred calves for veal and light-weight beef production are recommended. If a pasture shortage necessitates a reduction in crossbred numbers carried beyond the veal stage, steers should be selected for retention preferentially over heifers.

REFERENCES

BLAIR, M. M. 1944: "Elementary Statistics". Holt, New York.

BRODY, S. 1945: "Bioenergetics and Growth". Reinhold, New York.

KIDWELL, J. F. 1954: Some Growth Relations in Range Cattle. *J. Anim. Sci. 13:* 54-60.

KNAPP, BRADFORD, JR.; CLARK, R. T. 1950: Revised Estimates of Heritability of Characteristics in Beef Cattle. *Ibid. 9:* 582-7.

KOGER, M.; KNOX, J. H. 1951: The Correlation between Gains Made at Different Periods by Cattle. *Ibid. 10:* 760-7.

LUSH, J. L.; JONES, J. M.; DAMERON, W. H.; CARPENTER, O. L. 1930: Normal Growth of Range Steers. *Texas agric. Exp. Sta. Bull. 409.*

MACDONALD, M. A.; BOGART, R. 1955: Relationship Between Rate and Efficiency of Gain and Type in Breeding Beef Cattle. *N.Z. J. Sci Tech. A36:* 460-9.

MCMEEKAN, C. P. 1954: Good Rearing of Dairy Stock. *Proc. Ruakura Farmers' Conf. 1954:* 186-233.

——————1956: Crossbred Beef. *Ibid. 1956:* 101-14.

RADFORD, P. 1954: Producing Quality Beef from Dairy Stock. *Ibid. 1954:* 125-39.

URICK, J.; FLOWER, A. E.; WILLSON, F. S.; SHELBY, C. E. 1957: A Genetic Study in Steer Progeny Groups During Successive Growth Periods. *J. Anim. Sci. 16:* 217-23.

WILLIAMS, C. M. 1956: Observations on the Body Weight Change of Beef Calves. *Proc. Canad. Soc. Anim. Prod. (W) 4:* 1-6.

WINCHESTER, C. F.; ELLIS, N. R. 1956: Delayed Growth of Beef Cattle. *U.S. Dep. Agric. Tech. Bull. 1159.*

WINCHESTER, C. F.; HOWE, P. E. 1955: Relative Effects of Continuous and Interrupted Growth on Beef Steers. *U.S. Dep. Agric. Tech. Bull. 1108.*

BLOAT IN CATTLE

VIII. PREVENTION BY PASTURE SPRAYING WITH ANTI-FOAMING AGENTS. PRELIMINARY EXPERIMENTS WITH NON-LACTATING DAIRY COWS AND SHEEP

By C. S. W. Reid, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North

Appendix by R. W. Brougham[1], A. C. Glenday[2], and C. S. W. Reid

(*Received for publication, 12 December 1957*)

Summary

Pasture spraying with emulsified peanut oil, emulsified tallow, cream, or Avlinox (a ricinoleic acid derivative), provided a high degree of protection from bloat to non-lactating, monozygotic twin dairy cows. The application rates for a two-hour grazing period (equivalent to half the daily intake) were 25–30 ml oil, 30 g tallow, 15–60 g butterfat or 10 ml of Avlinox per cow.

Sprays containing a polymerised dimethyl silicone, used at rates from 1–4 g per cow, were ineffective.

Two methods of application of emulsified peanut oil were used: (a) strip spraying where the oil was applied to a narrow strip which was fed off before the unsprayed remainder of the ration was given; (b) total ration spraying where the oil was applied evenly over the whole ration, which was given at the one time. Both methods gave good control of bloat.

Moderate rain partly washed off sprays containing Avlinox or cream.

Herbage strips heavily sprayed with emulsified peanut oil were mildly repellent to some cows, while herbage sprayed with Avlinox was mildly repellent to all cows. Emulsified peanut oil, emulsified tallow, cream, and silicone had no obvious effect on palatability when sprayed over the whole ration.

In a single trial with grazing sheep, Avlinox sprayed on the pasture gave protection from bloat for several hours.

Marked but apparently temporary damage to the herbage was caused by the heavier application of emulsified peanut oil in strip spraying. Little or no damage was caused by the oil or any of the remaining agents when sprayed over the whole ration. An investigation of the effect of peanut oil sprays on the growth of red clover is reported in the Appendix.

INTRODUCTION

Earlier work at this laboratory showed that small amounts of anti-foaming agents, particularly those of an oily or fatty nature, given before feeding, would protect stall-fed cows from bloating on potent clover or ryegrass herbage (Johns 1954; Reid and Johns 1957). The duration of protection was short unless high dose rates were used. Other workers have also found anti-foaming agents to be of value in bloat prophylaxis (Smith—see Marston 1953; Nicholls 1954; Johnson *et al.*

---

[1]Grasslands Division, [2]Applied Mathematics Laboratory, Department of Scientific and Industrial Research, Palmerston North

N.Z. J. agric. Res. 1: 349–364.

1956; Barrentine B. F. pers. comm.) while Ferguson and Terry 1955) reported that anti-foaming agents prevented bloat in sheep dosed with extracts from potent lucerne.

The central problem in exploiting the protection afforded by anti-foaming agents is to find a practical method of maintaining effective amounts in the reticulo-rumen over the period of ingestion of dangerous herbage. This is a matter of some difficulty. The contents of the reticulo-rumen change continually as saliva, drinking water, and fresh feed are added, and liquid digesta flow out. The magnitude of this turnover in the cow is indicated by the figure of 150–170 l. calculated for the daily outflow from the abomasum (Phillipson 1956), i.e. 3–4 times the volume of ingesta normally present in the first two stomachs (approx. 40 l., Blamire 1952).* Only water-insoluble solid materials will remain in the rumen for any length of time, for these must first be broken down to small-sized fragments before they can pass on. Water-soluble or water-dispersible materials will be rapidly diluted. Transit of oils appears to be particularly rapid, probably on account of their water-imiscibility and low specific gravity.

Three possible solutions to the problem present themselves:

(a) the use of dose rates high enough to counter the losses between dosings;

(b) the absorption of the agent on to a solid vehicle which will disintegrate or be digested slowly in the reticulo-rumen, thus releasing the absorbed agent over a long period; and

(c) the frequent administration of small doses or, ideally, a continuous intake of the agent.

For (a) and (b), administration at milking is envisaged, the interval between dosings being 9–15 hr, while (c) implies administration in the field.

Neither of the first two possibilities appears to offer a practical solution. In the case of oil drenches, Reid and Johns (1957) found that whereas 50 ml protected stalled cows during the 2-hr feed immediately following administration, more than 10 times this amount was required to extend complete protection to the next 2-hr feed, a total period of 6 hr. On this basis, the dose rate required to protect a cow from one milking to the next would be uneconomic, possibly harmful to the animal (Buschmann 1930; Leroy 1949), and certainly deleterious to the milk and butterfat (McDowall *et al.* 1957). Similar objections can be raised to the use of surface-active agents such as detergents, high concentrations of which are likely to affect rumen organisms (Oxford (1952) found 0.2% Teepol XL killed all protozoa *in vitro*).

Reid and Johns experimented with a number of possible vehicles, including chaffed hay, meal, bran, wheat flour, sawdust, activated charcoal and diatomaceous earth. None significantly increased the duration of protection. Even if a suitable vehicle were found, however, administration itself would still present difficulties. A large proportion of a herd may refuse dry feed offered in the bail, especially when palatable green feed is plentiful, while the alternative, individual dosing, is most laborious.

---

* Assuming that water absorption in the omasum is balanced by secretion in the abomasum (Phillipson 1956).

The third possibility, that of the frequent administration of small amounts, is the most promising. Unfortunately, provision in the field of treated hay or licks, the simplest means of administration, cannot be relied upon because of the irregularity with which animals will take such materials, particularly in the presence of attractive green feed. Two more feasible measures are the addition of the anti-foaming agent to the drinking water or the application of the agent directly to the herbage. Of the two, treatment of the water supply appears the less reliable because of the wide variation in drinking pattern from animal to animal and in the same animal from day to day (Campbell and Munford 1957). Water intake is markedly affected by factors such as the ambient air temperature, the dry-matter intake, the dry-matter percentage of the feed, the level of milk production, and stock management practices (see Leitch and Thomson 1944; Winchester and Morris 1956; Campbell and Munford (in press)). At times, particularly in cool, wet weather, consumption may fall to very low levels. A practical limitation is that the method may be used only where there is no access to untreated water such as springs, streams, or puddles.

In contrast, application of the agent to the herbage has the advantages that the animal has little or no option as regards intake of the agent, and the latter is taken in when it is most needed, i.e. during the ingestion of bloat-producting pasture.

The simplest method of applying the agent to the herbage is by spraying. The present paper gives the results of preliminary trials made with non-lactating monozygotic twin dairy cows, and in which five anti-foaming agents and two systems of application, strip spraying and total ration spraying, were tried. A single trial with grazing sheep is reported and details are given in the Appendix of an investigation of the effects of peanut-oil sprays on the growth of red clover. A subsequent paper (Reid, in preparation) gives the results of experiments with lactating cows, and of farm trials. Practical details of the technique evolved for field use have already been published (Reid 1955).

METHODS

Pasture

The experimental area was an almost pure stand of red clover (*Trifolium pratense* L., 85–95%), other species present being mainly perennial ryegrass (*Lolium perenne* L.) and white clover (*T. repens* L.). The height of the herbage was 6–12 in. at the commencement of the trials (28 March) and 15–18 in. at the end (3 May 1955).

In late spring, the area had been mown for silage, but regrowth had been slow owing to a dry summer. No bloat occurred in cows introduced to graze some of the area in early March. The pasture became dangerous in the last week of March after vigorous growth had taken place following warm rains. The potency remained high during the first four weeks of the trials and declined slowly thereafter. Even in the most dangerous period, however, there were occasional days on which little bloat occurred.

Table 1 shows the maximum bloat scores recorded during three mornings' grazings in the first week of the trials and two mornings' grazings in the fourth week. No preventive treatments were carried out on these occasions. However, a number of bloated animals had to be drenched for safety. Since these moderately or severely affected animals would otherwise have become dangerously bloated, the grades of bloat given in the table do not represent the full potency of the herbage.

TABLE 1. OCCURRENCE OF BLOAT ON EXPERIMENTAL AREA WHEN NO PREVENTIVE TREATMENT USED[1]

Date	28.3.55	30.3.55	1.4.55	20.4.55	26.4.55
Total number of cows	8	8	8	8	8
Number of cows with bloat scores[2] of:					
Nil	1		2		
I					
II	4	3	1	3	
III	3	3	5	4	5
IV		2		1	3
Number of cows drenched[3]	1	4	3	2	6

[1] Data from a two-hour grazing period on the morning of each day.

[2] Bloat scoring as described by Johns (1954) I—Mild; II—moderate; III—severe; IV—dangerous.

[3] Drenches were administered to dangerously bloated cows, and to those which would have become so if left untreated. The drenches contained anti-foaming agents, usually peanut oil, but emulsified tallow or cream were given on occasions.

Animals

Five sets of monozygous twins and a single cow were used. Their susceptibility to bloating had been well established in previous work at this laboratory (Johns 1954; Reid and Johns 1957). Not all animals were used in every trial, the actual number varying from eight to eleven.

In any one trial, the members of each twin set were divided between the treatment and the control groups. Selection procedure was as follows: Each morning one member of each of the available sets was picked at random to form the treatment group. The remaining members of the sets formed the control group. In the afternoon the complete groups were reversed, the morning's control group becoming the afternoon's treatment group and vice versa.

Grazing

There were two two-hour grazing periods each day.

For each grazing, a ration of fresh pasture equivalent to approximately 5.0 kg dry matter per cow was offered. This allowance was slightly in excess of the needs of appetite. If no bloat occurred, the area, apart from soiled patches, was close- but not hard-grazed. If bloat did occur, a substantial proportion of the ration was left untouched.

To provide extra foot room, the experimental plot included along one side of the ration a mown area, the herbage from which was raked off (Fig. 1).

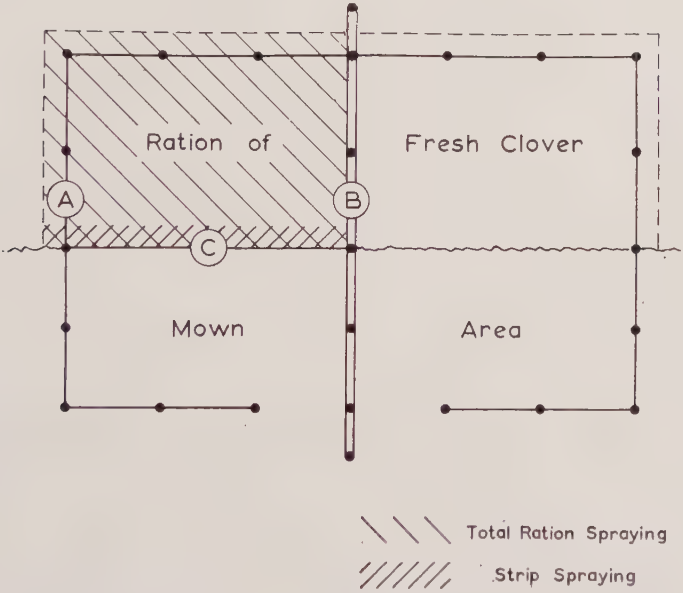


Fig. 1.—Areas sprayed in strip spraying and in total ration spraying experiments. A—single strand perimeter fence, set in about 1 yd from edge of clover ration (dashed line); B—double strand dividing fence; C—single strand fence along near edge of ration (used only in strip spraying—see text).

The plot was surrounded by a single-strand electric fence (A, Fig. 1). To prevent the animals from obtaining extra feed by reaching under this fence, either the latter was set in about one yard from the edges of the ration as in Fig. 1, or a strip three feet wide was mown around the outside and raked off. The plot was divided in two by a double-wire electric fence (B, Fig. 1), and one half was used for the spraying trial and the other as the control area.

When not grazing, the animals were kept in a bare, sheltered yard with access to water.

Sprays

Five anti-foaming agents were tested:

Peanut oil—a refined commercial product of food grade;

Avlinox*—a proprietary bloat remedy, a polyoxyethylene derivative of ricinoleic acid;

Tallow—an aqueous emulsion containing 33% W/V of mixed beef and mutton tallows, with soap and glyceryl monostearate as emulsifying agents (Emulsified Tallow A of Reid and Johns 1957)†;

Cream—a pasteurised sample containing 35% W/W butterfat;

DC Antifoam AF—a water-dispersible emulsion containing 29% W/V of a semi-solid polymerised dimethyl silicone‡;

Sprays containing Avlinox, emulsified tallow, cream, or the silicone were prepared by diluting the measured quantity of agent with water to a final volume of 1.5 l.

Peanut-oil sprays were prepared by emulsifying the oil in water, the emulsifying agent being a non-ionic detergent, Lissapol NX (a polyethylene glycol derivative)*. Emulsification was carried out by mixing for 5 min in a Waring Blendor the following proportions of the ingredients:

Peanut oil	100–150 ml
Lissapol NX (10% soln.)	10– 15 ml
Water	400 ml

The resulting emulsion was diluted further to a final volume of 1.5 l.

The stability of this spray was adequate for the purpose intended as there was little breakdown after standing for 2 hr. On one occasion, 10 g of Cellophas B (sodium carboxymethyl cellulose)* was used instead of Lissapol NX. This material had to be dissolved in warm water.

All sprays were made up immediately before use.

Spraying

Spraying was carried out using a knapsack sprayer and hand wand with two low-volume, size 33, solid-cone nozzles.

Two distinct systems of application were used:

1. Strip spraying.
2. Total ration spraying.

In strip spraying, the spray was applied to a narrow strip about 3 ft wide along the near edge of the ration. The cows were offered this strip first, and, to graze it, they had to reach under an electric fence (C, Fig. 1). When they had eaten down the sprayed strip, this electric fence was removed and they were allowed to graze the remainder of their ration for that particular grazing. The main precaution necessary was to reduce competition by providing an adequate length of sprayed strip and an adequate amount of foot room.

---

* Supplied by the distributors, Imperial Chemical Industries (N.Z.) Ltd.

† Prepared and supplied by W. and R. Fletcher (N.Z.) Ltd.

‡ Manufactured by the Dow Corning Corporation, U.S.A. Sample supplied by the agents, Swift and Co. Ltd., Wellington.

In total-ration spraying the spray was applied as evenly as possible over the whole of the ration allowed for the particular grazing. The whole of the ration was given at the one time. The main precaution necessary was to ensure that all the feed within reach of the animals was sprayed.

In both cases, spraying was carried out immediately before grazing.

RESULTS

Emulsified Peanut Oil

Emulsified peanut oil was used to investigate strip spraying and total-ration spraying as well as to determine its suitability as a material for bloat control. The results of the 12 trials with this anti-foaming agent are summarised in Table 2. The dose rates used were 25 ml of actual oil per cow in strip spraying, and 30 ml per cow in total-ration spraying.

TABLE 2. COMPARISON OF STRIP SPRAYING AND TOTAL-RATION SPRAYING WITH EMULSIFIED PEANUT OIL FOR PREVENTION OF BLOAT

Method	Strip Spraying		Total-ration Spraying	
Numbers of Trials	6		6	
Groups	Control	Spray	Control	Spray
Dose rate, ml/cow	—	25	—	30
Number of cow-feed units[1]	25	23	24	24
Number of cows with bloat scores[2] of:				
Nil	6	21	15	23
I	4	2	5	1
II	12		3	
III	3		1	
Number of cows drenched[3]	2			

[1] A cow-feed unit was one cow grazing for two hours.

[2] For classification, see footnote, Table 1.

[3] Drenched 50 ml peanut oil.

There was little difference in effectiveness between the two methods of application, both providing a highly significant control of bloat. (Chi^2, group Nil *versus* groups I–III, was 19.60 for strip spraying, and 6.19 for total-ration spraying, each for 1 d.f.).

In strip-spraying trials, some cows were reluctant to eat the heavily sprayed strip. On two occasions, the cows concerned subsequently bloated to grade I. There was never any reluctance to graze the areas where the total ration was sprayed.

Minor degrees of bloat often occurred in the afternoons where the total ration was sprayed. This was probably related to the fact that the treatment group in the afternoon was the morning's control group. The rate of intake of oil may have been too slow to prevent completely foaming of the fresh feed on top of a fill of bloat-producing feed from the morning. In contrast, in the strip-spraying regime, animals that were "puffy" when first offered the sprayed strip rapidly deflated as they ate the treated herbage. This was repeatedly observed in the subsequent trials with lactating cows. Undoubtedly it was due to the ingestion of the dose of oil over a short interval, resulting in a high initial concentration of the anti-foaming agent in the rumen.

Peanut oil emulsified with Lissapol sprayed easily, but when emulsified with Cellophas it required considerably more effort to apply because of its viscosity. At a higher dilution, or with power spraying, this would probably not be of consequence. The oil and Cellophas emulsion was effective.

Light rain fell during the times of spraying and of grazing in two total-ration-spraying trials, but did not affect the degree of protection given by the oil.

Total-ration spraying, in which the oil was spread over a large area, caused little visible damage to the herbage. On close examination, green translucent spots from 0.5–2.5 mm in diameter were found to be present on some of the leaves. The heavy rate per unit area used in strip spraying, however, caused marked damage, particularly when the weather was hot and sunny. Whole leaves became translucent and sometimes mild wilting occurred. However, even when damage was heavy, it did not appear to affect subsequent regrowth. An investigation of the effect of peanut-oil sprays on the growth of red clover is reported in the Appendix.

The remainder of the trials deal only with total-ration spraying.

Avlinox

The combined results of two total-ration-spraying trials with Avlinox are given in Table 3.

Avlinox proved an effective agent. However, it was noticeable that the animals did not appear to relish herbage sprayed with this material.

In one trial, light rain fell during spraying and the early part of grazing, and on this occasion three animals bloated to grade I. In the second trial, carried out in fine weather, one animal bloated to grade III. There was no obvious explanation for this.

Avlinox was easy to spray. It did not cause any visible damage to the herbage.

Emulsified Tallow

The combined results of two total-ration-spraying trials with emulsified tallow are given in Table 3. The dose rate aimed at was 30 g of actual tallow per cow. However, considerable difficulty was

TABLE 3. Combined Results of Trials with Emulsified Peanut Oil, Avlinox, Emulsified Tallow, and Cream as Pasture Sprays for Prevention of Bloat

Material	Emulsified Peanut Oil		Avlinox		Emulsified Tallow		Cream		All Sprays	
No. of Trials	12		2		2		7		23	
Groups	Control	Spray	Control	Spray	Control	Spray	Control	Spray	Control	Spray
Dose rate	—	25–30 ml actual oil/cow	—	10 ml/cow	-	30 g actual tallow/cow	—	15–60 g butterfat/cow	—	(see under individual sprays)
Number of cow-feed units[1]	49	47	10	10	8	8	29	28	96	93
Number of cows with bloat scores[2] of:										
Nil	21	44		6	1	7	7	24	29	81
I	9	3		3			5	2	14	8
II	15		4		5	1	5	1	29	2
III	4		6	1	2		11	1	23	2
IV							1		1	
Number of cows drenched	2		5				4		11	

---

[1] For definition, see footnote Table 2.

[2] For classification, see footnote Table 1.

experienced in spraying this material as small aggregates of tallow repeatedly blocked the nozzle strainers. The actual dose rates were therefore rather lower than the figure given in the table.

Despite poor spraying, tallow was effective.

Cows readily ate the sprayed herbage. The spray did not cause any visible damage to the plants.

Cream

The combined results of seven total-ration-spraying trials with cream are shown in Table 3. The dose rates used varied from 15–60 g of butterfat per cow.

TABLE 4. RESULTS OF TRIALS WITH DC ANTIFOAM AF AS A PASTURE SPRAY FOR PREVENTION OF BLOAT

Quantity of Silicone per Cow[1]	1 g		2 g		4 g		1–4 g	
Number of Trials	1		2		1		4	
Groups	Control	Spray	Control	Spray	Control	Spray	Control	Spray
Number of cow-feed units[2]	4	4	8	8	4	4	16	16
Number of cows with bloat scores[3] of:								
Nil		2	1	1			1	3
I			6	2	2	2	8	4
II	4			3	1	2	5	5
III		2	1	2	1		2	4
Number of cows drenched	1	2	1	2	1		3	4

[1] DC Antifoam AF contains 29% actual silicone.
[2] For definition, see footnote Table 2.
[3] For classification, see footnote Table 1.

A relationship between the weather conditions and the degree of protection afforded by cream sprays was evident. In dry weather, cream sprays gave protection equal to that provided by the emulsified peanut-oil sprays, but the effectiveness was markedly reduced by moderate rain, even when double the dose rate was used. In one trial carried out under the latter conditions, of the four cows on the sprayed area one did not bloat and the remaining three bloated to I, II, and III respectively. This effect appeared to be due to washing off of the cream.

The cows ate the sprayed areas readily. No visible damage to the herbage was caused.

DC Antifoam AF

The results of four total-ration-spraying trials with the silicone, DC Antifoam AF, are given in Table 4. The dose rate varied from 1–4 g of actual silicone per cow.

The silicone spray was ineffective, even when applied at the rate of 4 g of silicone per cow. This failure was the more marked because the potency of the clover was waning at the time of the experiments. During the first trial (1 g of silicone per cow) three bloated cows, two from the sprayed area, were drenched with 1 g of silicone. In each case, the drenches seemed to be so slow in acting as to leave little doubt that the silicone was ineffective. Two animals took respectively 105 min and 70 min to deflate from grade III to II and the remaining cow deflated from II to I in 60 min. To determine whether "frothy" bloat was still present, on the following day three bloated cows, two again from the sprayed area (2 g of silicone per cow), were drenched with 50 ml of peanut oil. The response of these animals (average deflation time III—0, 30 min) showed that no change in the type of bloat had occurred.

The cows ate the silicone-sprayed pasture readily. No visible damage to the herbage was caused by the silicone.

Avlinox Trial with Sheep

A single trial was carried out with grazing sheep, using Avlinox. The area used consisted of a number of plots each of 1/20 acre, the pasture being red-clover-dominant and 4–12 in. high. On the day before the trial, severe bloat had occurred in a flock of sheep introduced to this area to graze it down, and several deaths occurred before the animals were removed for safety. Eight of the plots were sprayed with 50 ml of this material and 8 left unsprayed as controls. The animals were introduced at 9.30 a.m. and left to graze all day. As will be seen from Table 5, marked protection was afforded by the spray. After 4 hr, this protection appeared to be waning, but even after 6 hr, it had not disappeared completely. To some extent, this wearing off of effectiveness appeared to be correlated with grazing behaviour, the animals tending to graze first the upper layers, i.e. the more heavily

TABLE 5. OCCURRENCE OF BLOAT ON SPRAYED AND ON UNSPRAYED PLOTS IN AVLINOX TRIAL WITH SHEEP

Plot Treatments	Total Number of Sheep[1]	Cases of Bloat[2] at Intervals after Depasturing						
		0.75 hr.	1.5 hr.	2.0 hr.	4.0 hr.	5.25 hr.	6.0 hr.	6.75 hr.
Fully sprayed	72	0	0	1	4	11	7	20
Nil	72	12	13	16	27	34	32	28

[1] There were 9 sheep in each plot.

[2] No attempt was made to grade bloat.

sprayed herbage, before grazing more closely to the ground. No attempt was made to assess the degree of bloat, but several animals in the control areas were very distressed. All recovered when removed from the clover.

As a test of the repellency of the spray, a further 4 plots were sprayed over one half only, 25 ml of Avlinox being used. That the spray was mildly repellent was indicated by the animals' preference for the unsprayed portions of these plots, but this effect had disappeared by the end of the first hour.

From the above experiment, it is clear that spraying anti-foaming agents will control bloat in sheep. However, the value of spraying as a method of prophylaxis may be limited by the effects of the grazing behaviour of these animals.

DISCUSSION

The possibility of spraying an anti-foaming agent on the pasture to prevent bloat in grazing animals was first suggested by Clark and

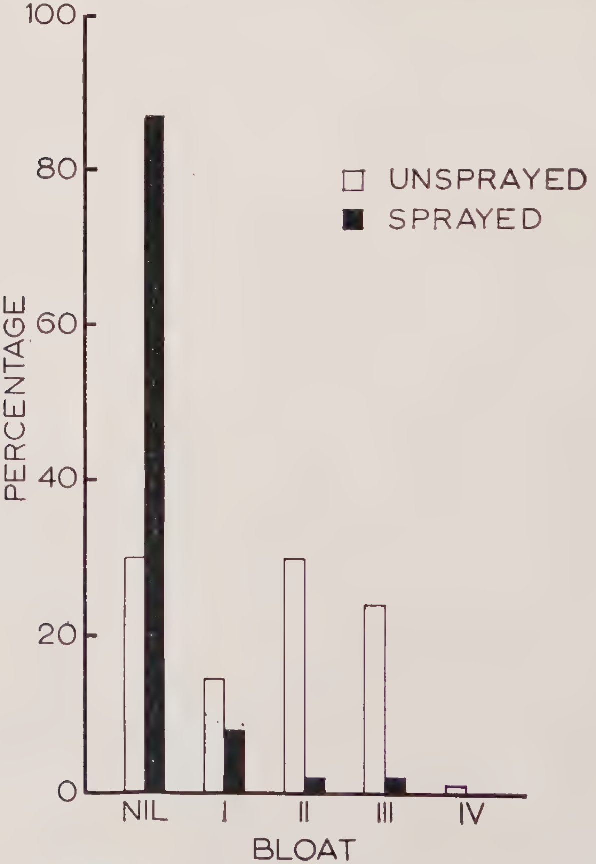


Fig. 2.—Distribution of bloat in cows grazing sprayed and control (unsprayed) plots. Combined results of trials with emulsified peanut oil, Avlinox, emulsified tallow, and cream (Table 3).

Weiss (1952). No experimental evidence was published in support of the idea. The results presented here, and in the succeeding paper (Reid, in preparation), demonstrate that pasture spraying is in fact a practical means of prophylaxis for bloat. In the present trials, of the five anti-foaming agents used, only one, the silicone DC Antifoam AF, failed to give protection. Against this failure (see below), sprays containing emulsified peanut oil, Avlinox, emulsified tallow, or cream, were all effective. In Fig. 2, the combined results of the 23 trials made with these four materials (Table 3) have been expressed as a percentage histogram. Despite the inclusion of the results of trials adversely affected by weather, the figure shows clearly the extent of the protection derived from sprayed anti-foaming agents. An index of the significance of these results is given by the high figure of 60.7 for 1 d.f., from a chi-square test of grade Nil *versus* grades I–IV.

Individually, the performance of each anti-foaming agent as a prophylactic spray was similar to its behaviour as a prophylactic or as a treatment in the stall-feeding experiments carried out by Reid and Johns (1957). Using the same cows as in the present trials, these authors had found peanut oil, emulsified tallow, and cream to be effective for either purpose. Avlinox was also effective, although there were indications that the doses used (10–15 ml) were minimal. Drenches of the silicone DC Antifoam AF, however, had proved unreliable for either prevention or treatment of bloat.

Variable results with silicones have been obtained by other workers also. Thus Hungate *et al.* (1955) found that a dose of 20 g given before a morning's grazing protected only 7 out of 10 steers that morning and that protection did not extend to the afternoon's grazing. Silicones are usually excellent anti-foaming agents, and compared with the amounts used in other foaming systems, the quantity used by Hungate *et al.* was large. A likely explanation of the irregularity seemed to be poor dispersion in the rumen. As a coating on the herbage, however, the silicone should be spread through the rumen mechanically, and the material was used as a spray in the hope that this dispersion might be sufficient for the agent to be effective. That it was not so suggests that dispersion may not be the only factor concerned.

Because of the intimate mixing of feed and anti-foaming agent, particularly when total-ration-sprayed areas were grazed, it was expected that only minimal amounts of the agents would be required to control foaming in the rumen. For this reason, with the exception of the silicone, the quantities used were based on the smallest quantities used by Reid and Johns (1957) in their stall-feeding experiments, i.e. 30 g of fat or 10 ml of Avlinox. These small quantities proved more than sufficient, since the average intake of anti-foaming agent by the cows was somewhat less than the amount allowed in calculating the rate of spraying. Losses during application, incomplete grazing of sprayed areas and variations in the grazing behaviour of individual cows all contributed to this.

The extent of wastage due to the first two causes differed with the two methods of spraying. Losses due to "run-off" from the leaves occurred in strip spraying, where the herbage was heavily coated, but were of only minor importance in total-ration spraying, at least at the relatively low pressures used here. Small amounts of sprayed herbage were left uneaten in some of the strip-spraying trials, but in total-ration spraying incomplete grazing was a consistent and important cause of waste. For, in calculating the ration to be given, allowances were made, based on previous experience, for trampling, soiling, and a small excess of feed beyond the needs of appetite, and all such areas were, of course, sprayed, but not eaten. There were differences in the effects of grazing behaviour also. Fast grazers obtained a larger proportion of strip-sprayed herbage than did slow grazers, while animals with large appetites obtained more of the anti-foaming agent from total-ration-sprayed herbage than did animals with small appetites.

Although the actual intake of the sprayed anti-foaming agent was thus not known, it is clear that in the case of oils and fats, the quantities required to control bloat are small in comparison with the total intake of crude plant lipid—of the order of 200 g per grazing in the present experiments. Since plant lipids have foam-breaking properties (Dr. J. L. Mangan, pers. comm.), it would seem possible that the ratio of foaming to anti-foaming agents in the herbage or the "availability" of the natural anti-foaming agents in the rumen, could be factors in the aetiology of bloat.

Strip Spraying **versus** *Total-ration Spraying*

From the results of the present trials, there would appear to be little to choose between the two methods of application with regard to the degree of protection afforded. However, because all the anti-foaming agent is taken during a short time interval, administration by strip spraying resembles drenching or the offering of treated dry feeds in the bail. As with the latter methods, the duration of protection is limited and it cannot be extended greatly without recourse to high dose rates. In the present trials, the grazing interval (2 hr) was too short to reveal this brevity of protection, but in subsequent experiments with milking cows (Reid, in preparation) it was plainly evident.

In other respects, there are wide differences between the two systems, strip spraying and total-ration spraying.

Strip spraying was designed to fit in with the system of grazing control using the electric fence to feed off small strips. It has the advantages that no calculation has to be made as to the ration of feed allowed, that application is easily carried out—a knapsack sprayer may be used if necessary—and that when bloat is already present in the herd, moderately bloated cows will be deflated if they eat their share of the treated strip. Against these advantages, however, any repellency of the spray is accentuated, and herbage damage is intensified. No protection is given to animals which, for any reason, do not eat the sprayed strip, e.g. because of lack of hunger, competition from other cows, or learning to wait for the unsprayed pasture.

In total-ration spraying, application is complicated by the need to calculate the area to be sprayed and to adjust the spraying rate accordingly. Spraying itself must be carefully carried out to ensure that all feed within reach of the animals is sprayed, and that it is covered evenly. For any but the smallest commercial herds, tractor-mounted-boom spraying equipment seems essential. Total-ration spraying has the advantages that repellency, herbage damage, and the consequences of animal behaviour are all minimal. The principal advantage, however, is that the animals are *always* grazing treated pasture, and it seems that only by this method can the full benefit of application of the agent to the herbage be obtained.

ACKNOWLEDGEMENTS

My thanks are due to Mr. A. C. Glenday for carrying out the statistical analyses and to Messrs. P. Vleig and L. Madgwick for technical assistance. The advice and encouragement of Drs. J. Melville and P. D. Sears is gratefully acknowledged.

This work was carried out using electric-fencing equipment kindly donated by W. and R. Fletcher (N.Z.) Ltd.

REFERENCES

BLAMIRE, R. V. 1952: *Vet. Rec. 64:* 493.
BUSCHMANN, A. 1930: *Tierernahrung I:* (Quoted by Williams, Cannon, and Espe 1943: *J. Dairy Sci. 26:* 241).
CAMPBELL, I. L.; MUNFORD, R. M. 1957: *Proc. N.Z. Soc. anim. Prod. 17:* 25.
——————1958: *J. agric. Sci.* (in press).
CLARK, R.; WEISS, K. E. 1952: *Fmg. S. Afr. 27:* 282.
FERGUSON, W. S.; TERRY, R. A. 1955: *J. agric. Sci. 46:* 257.
HUNGATE, R. E.; FLETCHER, D. W.; DOUGHERTY, R. W.; BARRENTINE, B. F. 1955: *Appl. Microbiol. 3:* 161.
JOHNS, A. T. 1954: *N.Z. J. Sci. Tech. A36:* 289.
JOHNSON, R. H.; BROWN, L. R.; JACOBSON, N. L. 1956: *J. Anim. Sci. 15:* 1294.
LEITCH, I.; THOMSON, J. S. 1944: *Nutrit. Abstr. Rev. 14*: 197.
LEROY, A. M. 1949: *Proc. 12th int. Dairy Congr. 1:* 21.
McDOWALL, F. H.; REID, C. S. W.; PATCHELL, M. R. 1957: *N.Z. J. Sci. Tech. A38:* 1054.
MARSTON, H. W. 1953: *Rep. Conf. Rumen Function. U.S. Dep. Agric., Washington D.C.*
NICHOLLS, R. E. 1954: *Proc. ann. Meet. Amer. vet. med. Ass.* p.178.
OXFORD, A. E. 1952: *J. gen. Microbiol. 5:* 83.
PHILLIPSON, A. T. 1956: *Proc. 7th int. Grass. Congr:* 229.
REID, C. S. W. 1955: *Proc. 17th Conf. N.Z. Grassl. Ass.:* p.70.
REID, C. S. W.; JOHNS, A. T. 1957: *N.Z. J. Sci. Tech. A38:* 908.
WINCHESTER, C. F.; MORRIS, M. J. 1956: *J. Anim. Sci. 15:* 722.

APPENDIX

THE EFFECT OF EMULSIFIED PEANUT OIL SPRAYS ON THE GROWTH OF RED CLOVER

By R. W. BROUGHAM, A. C. GLENDAY, and C. S. W. REID

Of the various sprays shown to be effective in controlling bloat, only those containing peanut oil caused any visible damage to the herbage.

To determine the severity of this damage on the growth of red clover (*Trifolium pratense* L.), a factorial mowing trial was carried out at the Grasslands Division, Palmerston North. Pure red-clover stands which had been allowed to grow to two heights, 4 in. and 9 in., were given three treatments (four replications of each): Nil (control), and spraying with emulsified peanut oil at 1 and 2 gal of oil per acre. The oil was emulsified with Lissapol NX as before. Following spraying, randomly sited portions of each plot were cut at alternate intervals of 4 and 3 days for a period of 3 weeks and D.M. yields measured.

Results

Visible damage of the kind described in the foregoing paper was evident immediately after spraying, the shorter clover plots sprayed at the higher rate being the most affected.

TABLE 6. THE EFFECT OF EMULSIFIED PEANUT-OIL SPRAYS ON THE GROWTH OF RED CLOVER
(Mean yields in lb D.M./acre)

Height of Clover when Sprayed (in.)	Treatment (gal/ac)	Cutting (Days after treatment)	
		4	7
4	Nil (Control)	1355 ± 81	1650 ± 165
	1	1135	1630
	2	1050	1590
9	Nil (Control)	3230 ± 81	4180 ± 165
	1	3170	4140
	2	3040	3890
F Ratio (2, 10 d.f.)		4.67*	0.63 n.s.

* = Significant at 5% level.
ns = Not significant.

The results obtained for the first two cuttings are shown in Table 6. A significant difference in yield between the control and the sprayed plots, indicating a reduction in growth rate due to the application of the oil, was obtained at the first cutting, made 4 days after spraying. This occurred with both heights of herbage and both rates of spraying, the heavier application having the most marked effect. At the second cutting, made 7 days after spraying, and at the remaining cuttings (11, 14, and 18 days) no significant differences were found.

In this trial, the herbage was leafy at spraying and the stands were left undisturbed until cut for yield measurements. Any effects due to spraying therefore should have been maximal. However, the results suggest that the damage was local and physical rather than a systemic phytotoxicity. The fact that heavy rain fell within 48 hours of spraying may have helped to reduce the persistency of the growth differences. If rain did have such an effect this would be a further indication of the superficiality of the damage. In farming practice the immediate grazing off of sprayed pasture would be an important factor in eliminating any marked decrease in growth rate.

THE EFFECT OF THYROXINE ON WOOL GROWTH AND LAMB PRODUCTION

By I. E. COOP, and V. R. CLARK, Canterbury Agricultural College*

(*Received for publication, 29 January 1958*)

Summary

In a series of trials over the period 1954–57 inclusive some 650 sheep of the Corriedale breed have been implanted with 1-thyroxine and the following observations made:—

i. The rate of wool growth is stimulated if the implantations are made during the late summer and autumn period (January-April) but implantations made in the winter, spring, and early summer (June-December) are ineffective.

ii. The optimum dose level appears to be in the region of 60–90 mg as a single implantation in sheep of 120–130 lb body weight.

iii. 90 mg implants cause an 8 lb loss in body weight over the first month, which is slowly but not fully regained in subsequent months.

iv. 90 mg thyroxine implanted at the optimum time gives on average a fleece weight increase of 0.6 lb (or 6%) the increase being mainly due to additional staple length. Fleece grade is not significantly improved, but the percentage of cotted fleeces is reduced.

v. Late-summer implantations made before mating for maximum wool response have caused a slight increase in barrenness and decrease in twinning, both statistically non-significant.

vi. No adverse effects other than those mentioned in (iii) and (v) have been observed.

vii. The practical implications of the use of thyroxine to stimulate wool production are briefly discussed.

INTRODUCTION

The first demonstration that the administration of 1-thyroxine to sheep could increase the rate of wool growth was made at this College by Hart (1954, 1955). The sheep used in these initial experiments were dry Corriedale ewes fed in pens on a constant diet. Responses were obtained to both injection and implantation but the latter proved to be the more useful method. A single implant caused augmentation of wool growth for a period of approximately four months, the increase being equivalent to 15% of annual fleece weight if a single 60-100 mg implant were used. Following implantation the sheep lost up to 10 lb of body-weight in the first few weeks but subsequently most of this was regained.

In 1955 Hart commenced a series of field trials using 60–90 mg implants in breeding ewes in a number of different flocks (mainly Romney) throughout the country. Increases in fleece weight ranging from 5-15% of annual fleece weight were obtained in these trials (Hart 1957). Furthermore in the Romney ewes employed in the trials there was an increase in staple length, and an improvement in commercial grading of as much as one half of a grade interval. The results of the thyroxine implantation on reproductive performance and lactation are being submitted by Hart for publication.

---

* Postal address: Lincoln College, Christchurch, New Zealand.

In the United Kingdom, Labban (1957) has recently shown that wool production can be very significantly increased by inclusion of thyroxine in the feed. The sheep were Suffolk ewe lambs employed over the twelve months following weaning. No loss of body weight was observed. This method of administering thyroxine is however not very practicable and the total amounts fed (3.5–5 mg per day) were twenty times greater than the implants used by Hart.

The present paper summarises the results of trials covering a four-year period from 1954–57 inclusive. They were conducted as a direct result of Hart's original observations and planned with his assistance. They were intended to occupy an intermediate position between his controlled-intake pen-feeding trial with small numbers of sheep on the one hand and his extensive field trials on the other.

Experimental Methods and Materials

The sheep employed were all of the Corriedale breed. They were run under normal grazing conditions on the Ashley Dene farm of the College.

In the first two years (1954–1955) wool growth was measured by clipping a delineated area at regular intervals as described by Coop (1953). Greasy weight, length, count, and clean scoured wool were measured. In analysing the data so obtained, inter-sheep variation in the level of wool production has been minimised by relating the post-treatment wool production of each sheep to its pre-treatment production from the same skin area, and, to achieve this, pre-treatment clippings were made for periods of several months before the implantations were made.

Sheep were individually identified by ear tag and lambs were identified and ear-tagged at birth.

The thyroxine pellets used were either of 20 or 30 mg, so that, for example, a 60 mg implantation was made with two 30 mg pellets both implanted in the one site at the one time. Unless otherwise stated, all implantations were single implantations, that is, made at one time. For the first three years, subcutaneous implantations were made in the brisket but in the last year they were made behind the shoulder.

Gains in fleece weight have been measured as either the actual annual greasy-fleece weight or as estimated greasy-fleece weight calculated from the clean scoured wool of the clippings and an assumption (which is known to be approximately correct) of 10 lb as an average annual greasy fleece weight.

Results

1954

At this early date little was known of the optimum dose level or of how much more variable the effects would be in paddock-fed sheep than in pen-fed sheep. Consequently the numbers of sheep used per treatment proved in many cases to be too small in relation to the responses obtained and the year's work can best be described as exploratory.

Pregnant Ewes

Two groups each of 20 four-year in-lamb ewes due to start lambing on 15 August were selected, weighed, randomised, and implanted on 24 May. One group of 20 ewes acted as control, and the other group was subdivided into four, each of five ewes, and implanted with 20, 40, 60, and 80 mg thyroxine. Liveweight losses of up to 7 lb (80 mg group) occurred in the first four weeks but were largely recovered by the end of the year.

Relative to the control group, wool production over the 11 weeks following treatment, that is until 10 August, was altered by − 5 per cent, − 10 per cent, + 7 per cent and + 8 per cent for the 20, 40, 60, and 80 mg groups respectively. From 10 August until weaning, differences were small. The maximum influence which these results could have on annual fleece weight is of the order of 0.1 lb or 1 per cent.

The thyroxine had no effect on the lambing or growth rate of the lambs.

Lactating Ewes

Eighty ewes all with lambs approximately two weeks old were randomised on 13 September. Some groups were implanted then, others on 27 October. The lambs were weaned on 25 November but clippings of wool from the ewes were continued at monthly intervals until 2 March 1955.

The age-corrected weaning weights of the lambs of all September-implanted groups were increased by 3 lb, insufficient to be statistically significant. The lambs from the ewes implanted in October (one month before weaning) were not affected.

The treatments and the estimated effect on annual fleece weights are given in Table 1.

TABLE 1. EFFECT OF THYROXINE IN LACTATING EWES

Date of Implantation	Dose (mg)	No. of Ewes	Maximum Body-weight Loss (lb)	Increase in Fleece Weight (%)
Control	Control	40	—	—
13 September	60	8	6	+ 2
13 September	100	9	10	− 1
27 October	60	10	3	− 2
27 October	100	8	12	+ 2
27 October	160	5	12	+ 2

The effect on wool production was small, irregular, and within experimental error. Short-term effects which would be discernible in individual monthly clippings were likewise very small and led to the conclusion that thyroxine does not influence the rate of wool growth in lactating ewes.

Dry Ewes and Ewe Hoggets Implanted in Spring

On 30 September, twenty-two dry ewes were randomised for treatment and clippings were made at monthly intervals until 2 March 1955. On 30 August twenty ewe hoggets were similarly treated. The results are given in Table 2.

TABLE 2. EFFECT OF THYROXINE IMPLANTED IN DRY EWES AND HOGGETS IN THE SPRING

Sheep	Date of Implantation	Dose (mg)	Maximum Live-weight Loss (lb)	Increase in Fleece Weight (%)
6 dry ewes	Control	—	—	—
5 dry ewes	30 September	80	3	+ 1½
6 dry ewes	30 September	120	9	− 5
5 dry ewes	Sept., Oct., Nov.	3 × 40	5	− 3
5 hoggets	Control	—	—	—
5 hoggets	30 August	80	8	− 1
5 hoggets	18 October	80	5	0
5 hoggets	Aug. & Oct.	2 × 40	2	− ½

Again one must conclude that these results provide no evidence for an increaase in rate of wool growth, neither do the short-term monthly clippings indicate any outstanding effect.

1955

During 1955 three series of implantations were made, the first in ewes in the late summer before mating, the second a repetition of the 1954 trial with pregnant and lactating ewes, and the third in wethers in the spring. From the lessons of the previous year fewer groups with larger numbers of sheep were used.

Ewes Implanted between Weaning and Mating

Throughout 1954 a considerable number of ewes had been clipped regularly as part of a wool-growth study. These ewes had been run together all the time and had not been treated with thyroxine. From these 54 four- and five-year ewes and eight two-tooth ewes were available for this particular trial and their 1954 production was used as a reference for thyroxine treatment. Each age-category of the ewes was separately randomised into the different treatments. The ewes were put to the ram on 17 March.

The results obtained are given in Table 3 and Fig .1.

The calculated gain in fleece weight is that calculated for a 10 lb fleece from the increased rate of growth shown in the monthly clippings

TABLE 3. Effect of Thyroxine Implanted Between Weaning and Mating

Group	No. Ewes	Date of Implantation	Dose (mg)	Max. Weight Loss (lb)	Weight loss after 9 months (lb)	Calculated gain in Fleece Weight (lb)	Actual Gain in Fleece Weight (lb)
I	19	Control	—	—	—	—	—
II (a)	8	19 Jan.	80	4	3	0.66	0.8
II (b)	8	19 Jan.	120	6	4	0.72	
III (a)	14	2 March	80	5	3	0.63	0.9
III (c)	14	2 March	120	7	3	0.71	

following treatment while the actual gain is taken from actual weights shorn at the end of the year.

The effect on rate of wool growth is shown in Fig. 1.

The 80 and 120 mg doses gave parallel responses in both groups II and III, the 120 mg being slightly the greater, so the two dose rates have been combined in order to simplify the curves shown in Fig. 1.

From these results and other observations made the following conclusions are drawn:

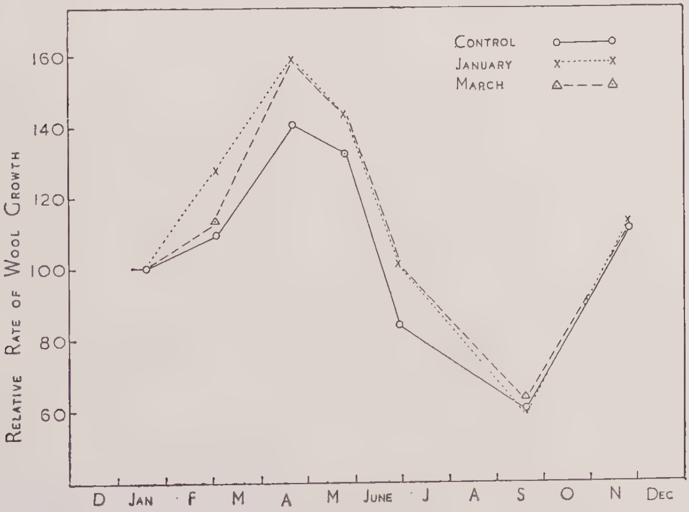


Fig. 1.—Relative rate of wool growth in ewes implanted in January and March.

Wool Growth

(a) The 120 mg doses gave only slightly greater gains than the 80 mg dose.

(b) The response lasts for a period between 5 6 months for January implants and between 4–5 months for March implants.

(c) The two-tooths gave similar responses to the older ewes.

(d) Growth rate is stimulated to the extent of approximately 15% for 2–3 months following treatment. During these months the increase in length amounted to 12% so that this increased length accounts for most of the weight increase.

(e) The increase in fleece weight is of the order of 0.6–0.8 lb.

(f) There was no improvement in the grade of fleece wool produced.

Reproduction. The performance of the January-implanted ewes was very similar to that of the control, one barren ewe in each, no differences in number of twins born or the mean date of lambing. The March-implanted ewes however seemed to be adversely affected, as five of the 28 proved to be barren and the mean lambing date was retarded by ten days. No differences in lamb survival or weaning weight were observed. The barren ewes were excluded from the analyses in Table 3 and Fig 1.

Ewes Implanted while Pregnant or Lactating

A line of 80 six-tooth ewes which had been mated with those in the above trial and subsequently run with them for the rest of the year was randomised on 20 April into four groups each of 20 ewes. All of these ewes had been run together since shearing in November 1954 and though they had not been clipped before, the five months' growth of wool clipped for the first time on 20 April was used as the pre-treatment reference.

The treatments were as follows:

Control group	—not implanted.
April group	—implanted on 20 April, half at 60 mg and half at 100 mg.
June group	—implanted on 29 June, half at 90 mg and half at 120 mg.
September group	—implanted on 14 September, all at 90 mg.

TABLE 4. Effect of Thyroxine Implanted in Pregnant or Lactating Ewes

Group	Maximum Live-weight Loss (lb) (90–120 mg)	Live-weight Loss at End of Year (lb)	Weaning Weight of Lambs (lb)	Calculated Gain in Fleece Weight (lb)	Actual Gain in Fleece Weight (lb)
Control	—	—	66.0	—	—
April	8	1	66.9	0.35	0.4
June	6	2	66.3	0.15	0.4
September	—	—	68.7	0.0	0.3

The mean lambing date of the ewes was 26 August and when the September group was implanted all the ewes had lambed and the average age of the lambs was 19 days.

The results obtained are summarised in Table 4.

In the case of the April and June implantations, the higher dose gave slightly higher wool response, but not commensurately so, so the dose-rate subgroups have been combined in both Table 4 and Fig. 2.

The weaning weight of the lambs has been corrected for age and multiple births. The calculated gain in fleece weight is that derived from the periodic clippings. The actual fleece weights given suggest that the wool responses are greater than those calculated from the clippings. With the numbers of sheep involved and the fact that the calculated gain is, as it were, corrected against pre-treatment wool growth, it is considered that the calculated gain is the more accurate.

The conclusions drawn from the above data and from other observations not included in Table 4 are as follows:

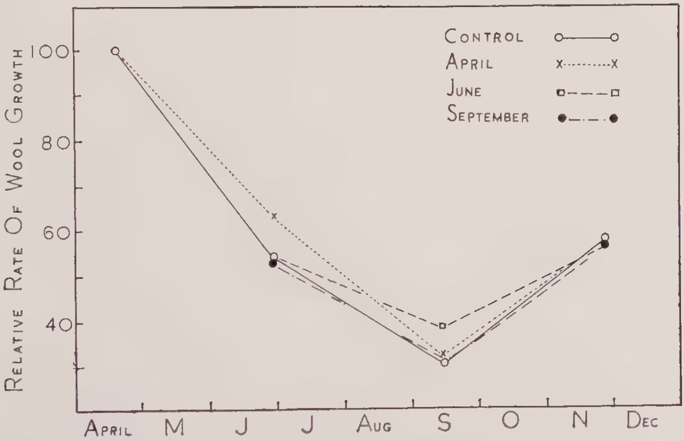


Fig. 2.—Relative rate of wool growth in ewes implanted in April, June, and September.

The higher dose rates gave the greater responses in calculated wool growth, e.g., April 60 mg 0.30 lb, 100 mg 0.40 lb, but the responses were less than proportional to dose rate.

Implantations made in the autumn (April) during early pregnancy caused a significant but small increase in wool growth (0.35 lb gain in a 10 lb fleece weight).

Implantations made in midwinter (June) during late pregnancy produced very little increase in wool growth.

Implantations made in September in lactating ewes failed to produce any increase in wool growth.

There was no significant change in fleece grade in any of the groups.

The weaning weight of the lambs from the ewes implanted after lambing was increased by 2–3 lb but this was insufficient to be statistically significant.

There were no effects due to treatment in respect to ewe mortality, lambing behaviour, and lamb survival.

Wethers Implanted in the Spring

Twenty-two four-tooth wethers were selected, weighed, clipped, randomised, and implanted on 23 September. The wool grown on the clipped area since shearing in 1954 was the pre-treatment reference standard. The sheep were weighed and clipped again on 17 October and finally on 25 November. The results are summarised in Table 5.

TABLE 5. Effect of Thyroxine on Wethers Implanted in Spring

Group	No. of Wethers	Live-weight Loss 23/9–17/10 (lb)	Increase in Wool Growth (%) 23/9–25/11
Control	9	—	—
60 mg	6	5	− 0.6
90 mg	7	8	+ 1.6

From the results it is clear that, as was the case with the spring implantations in ewes, there is the usual loss of body weight without any compensating increase in the growth of wool.

1956

Time of Implantation and Dose Rate Trial

The 1954 and 1955 trials had shown that thyroxine implanted from June to October was either ineffective or had very slight effect on wool growth, whereas implantations made in the period January-April definitely stimulated wool growth.

The present trial was designed to investigate two things:

Whether implantations made in ewes after weaning in December would be effective.

Whether smaller doses made during the responsive period would give measurable increases in wool growth.

For the trial, there were available at the end of 1955 a total of 82 two- to five-year-old ewes which had been clipped regularly during the previous twelve months and which had all been run together throughout the year. None of them had previously been treated with thyroxine. All had been weaned at the beginning of December 1955.

The ewes were randomised on the basis of age and live weight on 13 December 1955. One group of 11 ewes was implanted with 90 mg thyroxine. On 24 January 1956 all the ewes were re-weighed and clipped. Three groups each of 14 ewes were implanted with 30, 60, and 90 mg thyroxine. On 1 March the ewes were again weighed and clipped and a further group of 14 ewes was implanted with 90 mg thyroxine. Further clippings were made at intervals of 6–8 weeks throughout the remainder of the year.

The ewes were put to the ram on 14 March, lambs were weaned on 22 November, and the ewes were shorn the following day.

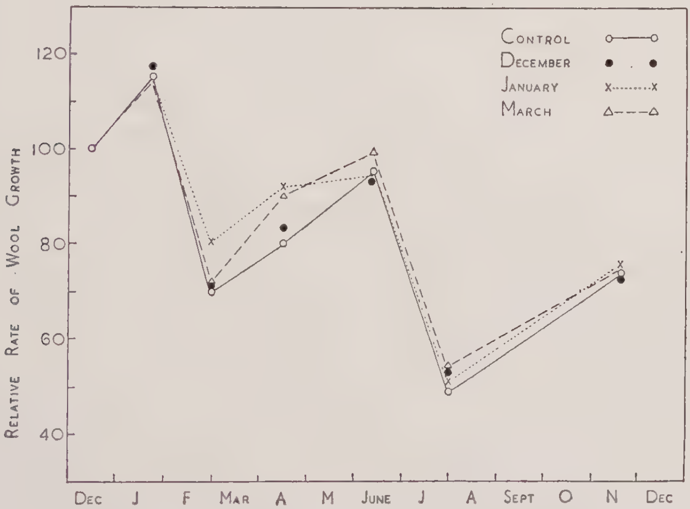


Fig. 3.—Relative rate of wool growth in ewes implanted with 90 mg thyroxine in December, January, and March.

The relative growth rate of wool for the 90 mg groups and the control are shown in Fig. 3.

An unusual feature of these curves is the fall in growth rate during February-March and the subsequent recovery. This was due to abnormal seasonal conditions. The summer of 1955–56 was the driest on record, the pastures were exceptionally bare, and even the control ewes were losing weight. The drought was broken at the end of March and the recovery in rate of wool growth was the response to improved nutrition in spite of the fact that normally the decline in growth rate sets in in March.

Nevertheless the effects due to thyroxine were not obscured. The figure shows that there was only a very small response from the December implantation, whereas the January and March implantations gave positive responses of approximately equal magnitude and of the same order as those of 1955. The January implantations had ceased to be effective by April (that is, after three months) and the March implantations by July (after four months). The clipping showed that 75% of the increased wool growth could be accounted for by increased length. Live-weight and wool-weight data are summarised in Table 6.

TABLE 6. EFFECT OF TIME OF IMPLANTATION AND DOSE RATE

Date of Implantation	No. Ewes	Dose (mg)	Maximum Live-weight Loss (lb)	Calculated Gain in Fleece Weight (lb)	Actual Gain in Fleece Weight (lb)
Control	15		—	—	—
13 Dec. 1955	11	90	7	0.05	− 0.1
24 Jan. 1956	14	30	6	0.35	0.0
24 Jan. 1956	14	60	6	0.45	0.4
24 Jan. 1956	14	90	10	0.63	0.5
1 Mar. 1956	14	90	6	0.64	0.8

The calculated gain in fleece weight from the clippings would be more accurate than the actual gain but both are of the same order of magnitude. The increase in fleece weight is largely due to increase in staple length. Mean count (as estimated visually and therefore subject to considerable error) was not affected, but grade was very slightly improved in all of the January and March implants.

In the group implanted in December, four ewes died within a month. These were the only sheep lost within 2–3 months of implantation out of the 650 implanted during the four years and it is concluded that this loss could have been fortuitous. The only dry ewes were two in the March group, while the January 60 mg. and 90 mg groups had fewer twins than the control but in other respects no differences in lambing and lamb survival were observed.

Fleece Weight, Grade, and Fertility Trial

The numbers of ewes employed in previous trials had been too small to place reliance on fleece weight, fleece grade and lambing results. It was for that reason that the clipping technique had been used to study wool responses. Accordingly in 1956, the more detailed study was supplemented by a field trial with a greater number of ewes.

On 20 February 1956 a line of 200 four-tooth ewes was randomised into two groups on the basis of fleece length, count, and style. One group of 100 ewes was implanted with 90 mg thyroxine and rams were put out with both groups 23 days later, on 14 March. These ewes had been pre-lamb-shorn in 1955 so that at the 1956 shearing they had 14 months' length of wool which could conceivably influence the classer's grading.

The mean fleece characteristics are summarised in Table 7. Commercial grade was scored as follows: AA 7, A 6, BB 5, B 4, C 3, D 2, E 1.

These results show that the thyroxine has increased fleece weight by 0.74 lb, a difference which is statistically significant, and length by 0.4 inches which can account fully for the increased weight. Yield was unfortunately not measured, but grader's estimate of yield was that there was no difference. Count remained unaltered. There were fewer cotted and broken fleeces in the treated sheep. The improved weight and length combined with fewer faults should have but did not lead to a noticeable improvement in commercial grade.

TABLE 7. Fleece Characteristics of Control and Thyroxine-implanted Ewes

	Fleece Weight	Count	Length (in.)	Grade	Cotted Fleeces %	Breaks %
Control	10.60	56.1	5.6	4.0	13	6
Implanted	11.34	56.0	6.0	4.1	4	0

The effect of the thyroxine on lambing performance is given in Table 8.

TABLE 8. Effect of Thyroxine on Lambing

Group	Barren Ewes %	Lambs Born (% of ewes lambing)	Mean Lambing Date
Control	4	116	19.2 Aug.
Implanted	7	113	20.6 Aug.

From these figures it appears that the thyroxine causes a slight adverse effect in respect to both barrenness and twinning but the differences are not significant statistically. Mean date of lambing was very slightly affected. No differences were observed in lamb survival and weaning weight.

1957

Fleece Weight, Grade, and Fertility Trial

A line of 630 ewes of mixed ages from four-tooth to five-year inclusive was randomised on 27 February on the basis of age into three groups and treated as follows:

90 ewes implanted with 60 mg thyroxine
110 ewes implanted with 90 mg thyroxine
430 ewes not implanted, for control.

These ewes were put to the Corriedale ram 14 days later, on 13 March. They were at all times run together in one mob.

The fleece records based on the 12 months' fleece shorn in December 1957 are given in Table 9.

Yield is estimated to 16% regain.

These results show that the thyroxine has again increased greasy fleece weight, the 60 mg by 0.27 lb which is not statistically significant and the 90 mg by 0.60 which is statistically significant ($P < 0.05$). Yield

TABLE 9. FLEECE CHARACTERISTICS OF CONTROL AND IMPLANTED EWES

	Fleece Wt. (lb)	Yield %	Clean Scoured Wt. (lb)	Count	Length (in.)	Grade	Cotted Fleeces %
Control	8.73	64.1	5.60	55.9	4.6	4.0	25
60 mg	9.00	65.7	5.91	56.0	4.6	4.1	21
90 mg	9.33	64.5	6.02	55.9	4.7	4.0	15

was not decreased by the thyroxine treatment so that the actual increase in clean scoured wool amounted to 5.5 and 7.5% of the 60 mg and 90 mg doses respectively. Grade was unaffected by treatment in spite of the percentage of cotted fleeces being reduced by the thyroxine. Length was not significantly affected.

The effect of the treatments on reproduction is summarised in Table 10.

TABLE 10. EFFECT OF THYROXINE ON LAMBING

Group	Barren Ewes %	Lambs Born (% of ewes lambing)	Mean Lambing Date
Control	2.8	123	19 August
60 mg	5.5	123	19 August
90 mg	2.7	117	18 August

These results show a slight increase in barrenness in the 60 mg group and a slight depression in twinning in the 90 mg group. Neither of these differences is statistically significant. The date at which the ewes lambed was not affected by treatment. A more detailed analysis of the lambing picture showed that in incidence of twinning the same relationship between the groups held at the beginning, middle, and end of lambing. In other words no significant short-duration effect of thyroxine was noted.

DISCUSSION

Body Weight

Following implantation of thyroxine, losses in body weight have invariably been observed at all seasons of the year. Our first weighings after implantation were usually made after an interval of 4–6 weeks and the loss at this stage is referred to as the maximum loss. This may not be the true maximum loss since Hart* has shown in penned sheep that there is an initial rapid loss of weight during the first two weeks followed by a slow regain. Nevertheless our observed losses are probably of the correct order. As might be expected the magnitude of the losses has varied somewhat with the nutritional conditions obtaining at the time.

---

* Paper presented at 32nd Meeting of Australian and New Zealand Association for the Advancement of Science, Section K (1957).

For implantations of 90 mg in sheep which usually weighed from 110–130 lb the mean loss was 8 lb with a range 5–12 lb. Implantations of 60 mg gave a mean loss of 6 lb. These weight losses are very slowly regained so that 6–12 months later the losses have been reduced to 2–3 lb.

Wool Production—Dose Level

Various dose rates have been used in the trials with an object of ascertaining the optimum level. The January-March implantations of 1955, 1956, and 1957 are relevant to this aspect of thyroxine treatment. They show that within the limits used, 30 120 mg administered in one dose, the stimulation to wool growth is greater the higher the dose level. Thus in 1955 80 mg gave a mean increase of 0.64 lb in annual fleece weight, and 120 mg gave 0.71. In 1956, 30, 60, and 90 mg gave respectively 0.35, 0.45, and 0.6 lb increases, while, in 1957, 60 and 90 mg gave 0.27 and 0.60 lb increases.

But in deriving the optimum level it is clear that body-weight loss must also be taken into account and this also increases with dose level so that the optimum must be a compromise. It is felt that this compromise lies in the 60–90 mg region. At levels higher than this, considerable body-weight losses are incurred and the increment of wool growth diminishes. At levels below 60 mg where weight losses are smaller and therefore more tolerable the gain in wool production drops to a level scarcely sufficient to compensate for the time and effort of implanting.

Wool Production—Time of Implantation

In Hart's original work the implantations and injections were made in the autumn and gave positive responses in wool growth.

In all of our experiments we have failed to obtain significant responses from thyroxine implantation at any time from May (early winter) to December (midsummer). Thus, in 1954, implantations made on 24 May in pregnant ewes produced either no responses at all or less than 0.1 lb. Later in the same year implantations in lactating ewes on 13 September and 27 October failed to increase wool growth, while implantations in dry ewes on 30 September and in ewe hoggets (approximately 12 months of age) on 30 August and 18 October likewise failed to produce any measurable response.

These observations were confirmed the following year when implantations in pregnant ewes on 29 June gave a slight positive (0.15 lb) response, in lactating ewes on 14 September and in wethers on 23 September no response at all. Finally, in the 1956 trial when one group of ewes was implanted just before the beginning of the year, on 13 December 1955, a doubtful positive response of less than 0.1 lb was obtained.

On the other hand, significant positive responses have been obtained in all implantations made from January through the late summer and autumn to April inclusive. For example, in 1955, implantations made on 19 January and 2 March gave increases of the order of 0.7 lb in fleece weight at dose levels of 80–120 mg. A group implanted on 20 April gave a significantly lower increase of 0.35 lb. In 1956, positive responses from 24 January and 1 March implantations yielded increases of 0.6 lb for 90 mg doses, while in 1957 implantations made on 27 February yielded increases of 0.27 and 0.60 lb for 60 and 90 mg doses respectively. These experiments show clearly that the wool growth response to thyroxine is highly dependent on the season of application.

This is in conflict with Labban's (1957) observations. He fed thyroxine continuously to ewe lambs from autumn (6 months of age) until nearly 18 months of age. He measured wool growth during the last months of treatment (corresponding to November, December, January in Southern Hemisphere) and found strongly positive responses in all of these months. No explanation can at present be offered to account for failure to obtain any responses during winter, spring, and early summer in contrast to positive responses in late summer and autumn. Hanneman *et al.* (1955) have shown thyroid secretion rates in sheep to be high in winter and low in summer, and to be increased by lactation but unaffected by pregnancy. While these observations may suggest a part explanation, it is clear that a great deal of fundamental knowledge must precede any explanation of thyroxine implantation effects. This is especially true as the sheep's thyroid gland displays some unusual features (Purves and Hart pers. comm.).

Wool Production—Fleece Characteristics

The results just discussed show that implantations of thyroxine made in the late-summer early-autumn period will in Corriedale sheep increase annual fleece weight by about 0.6 lb (6%). The response to thyroxine is very rapid and it lasts for approximately four months, as was found previously by Hart. The increased weight of wool has been shown in most cases to be due largely to increase in length since the measured increase in length is sufficient to account for most of the increase in weight, and since subjectively estimated count (related to diameter) remained unchanged although it must be recognised that visual estimations of count are subject to considerable error. In these respects our observations agree with those of Labban.

Increase in weight of fleece and length of staple are usually associated with increase in fleece grade. The work of Ferguson *et al.* (1949) and Coop (1953) has shown that the rate of wool growth is highly dependent on season of the year and it might be expected from this that the effect which an added increment of growth rate from thyroxine administration could make would be dependent on when the increment occurred. If it occurred during the period of minimum growth in winter and early spring (June September) it should give a more uniform fibre, fewer cotts and breaks and overall a higher grade. On the other hand if the increment had ceased before this period of minimum growth it could be ineffective in improving grade. Our experiments show that where a weight response was not obtained (May–December implantations) no improvement in grade resulted. Where a growth rate response was obtained (January–March implantations) there was either very little or no corresponding improvement in grade.

In the Romney flocks which Hart (1957) tested in his field trials there was a definite improvement in grade amounting to as much as one half of a commercial grade interval but in the one Corriedale flock tested the improvement was much smaller. Also in this flock the fleece weight increase was smaller than in the Romney. He has suggested that there could well be a breed difference in responses to thyroxine both in regard to the weight increase and grade improvement. Our observation certainly confirms that in the Corriedale any improvement in grade is either small or non-existent.

Lactation

It has been well established that administration of thyroxine or iodinated protein to dairy cows will increase milk production at the expense of body weight. Hart (1957) has shown a similar effect in ewes implanted with thyroxine after lambing, while Labban (1957) has confirmed that the feeding of thyroxine to the lactating ewe increases milk production and increased weaning weight of the lamb by about 5 lb. Our results have likewise confirmed the lactopoietic action of thyroxine. The thyroxine was implanted in the ewes at lamb-docking time when the mean age of the lambs was 2–3 weeks, and it resulted in increases in weaning weight of 3 lb in both 1954 and 1955. It is not proposed to discuss this further as the matter is being more fully dealt with elsewhere by Hart (in preparation).

Reproduction

In the course of these experiments approximately 650 sheep have been implanted with thyroxine in doses ranging from 30–160 mg. As a deliberate measure and as a test of possible farm use, no precautions were taken to maintain any standards of cleanliness, disinfectant was never used and instruments were not sterilised. With one exception, which has been mentioned earlier and can be discounted, no deaths have occurred which could be attributed to the thyroxine or to implantation.

The implantation of thyroxine into in-lamb ewes in the second, third, and fourth month of pregnancy has not resulted in any abortion or any noticeable effect on parturition and lamb survival.

The time-of-implantation study has shown that for maximum wool response the thyroxine should be administered in the January-March period which in general is before mating. The implantation of a massive dose of thyroxine might well be expected to have a profound effect on the metabolism and endocrine relationships of the animal, and it certainly takes nearly 10 lb of body weight off the average 120–130 lb ewe. Accordingly the first experiments in the autumn of 1955 were necessarily on a conservative scale and the results suggested a slight adverse effect. This tendency was confirmed in the 1956 time-of-application study. The larger-scale trial in 1956 with 100 ewes implanted on 20 February, 23 days before putting to the ram, showed a 7% drop in lambing percentage due both to increase in barrenness and fewer twins born. At this stage some of Hart's observations suggested that implantations should best be carried out about 12–14 days before joining with the rams. This was done in 1957 when 200 ewes were implanted on 27 February, 14 days before putting to the rams, but again there was a slight (4–5%) depression of lambing percentage due to both barrenness and twinning, though none of these differences was of sufficient magnitude to be statistically significant.

Hart (in preparation) has much more extensive data more especially with the Romney breed. It is proposed therefore that he should discuss our results along with his own in order to avoid unnecessary duplication. Suffice to say here that in our experience with the Corriedale breed small adverse effects each statistically non-significant have been obtained. More work is therefore needed in this breed to establish whether any effects are real and to determine the conditions under which a favourable or at least a not unfavourable response is obtainable.

The effect which thyroxine might have on the longevity and useful breeding life of the ewe is also an important aspect but this has not formed part of this study and at present there are no known facts about it.

On the practical side, these trials have shown that sheep are very tolerant to the large amounts of thyroxine implanted. It has been shown that in the Corriedale breed increases in wool production amounting to about 0.6 lb greasy wool in an annual clip of 10 lb per sheep (i.e. 6%) can consistently be obtained provided the thyroxine is implanted during the January–March period. There is little or no increase in grade but the percentage of cotted fleeces is decreased. On the question of body-weight loss there is the obvious implication that nothing is really gained by thyroxine implantation, the additional wool being obtained at the expense of body weight. The sheep must take in additional feed at some subsequent time to restore this loss and hence the efficiency with which wool is produced from pasture is not enhanced. But as Hart (1957) has already pointed out, there are conditions under which thyroxine could be used to aid efficiency of utilisation. The removal of excess body weight of ewes in the period from weaning to mating by shutting the ewes up on bare pasture is a problem in many areas. Thyroxine could obviously be used to advantage under these circumstances. Another condition not infrequently encountered is an abnormally bountiful summer and autumn pasture growth which cannot be efficiently utilised with the existing numbers of stock carried. On the other hand there are also seasons when the use of thyroxine could not be recommended. When feed is scarce or in only moderate supply, when carrying capacity is high, and especially when the standard of pasture control and efficiency of pasture utilisation is already high, thyroxine is not likely to increase the overall efficiency. The carrying of additional stock is more likely to increase the efficiency of pasture conversion than is thyroxine, except under the conditions already mentioned of the over-fat ewe or where there is a temporary feed surplus.

Another factor likely to govern the use of thyroxine at present is the effect on reproduction. Hart (in preparation) claims that lambing percentage can in fact be increased and certainly many (but not all) of his trials show this to be true, but until the conditions under which an increase and not a decrease can be even approximately defined the position remains unsatisfactory. The larger-scale trials (1956, 1957) reported in this paper showed a slight depression in lambing percentage which offset all the gains from wool. Perhaps, had the implantations been made 6–8 weeks before mating instead of 2–3 weeks, as was the case, this depression might not have been obtained. If one could ascertain the environmental conditions and the timing of implantation relative to mating so that even a slight increase in lambing percentage is obtained the usefulness of thyroxine would be very greatly enhanced.

ACKNOWLEDGEMENTS

The authors wish to thank the Department of Scientific and Industrial Research for financial assistance, and the Glaxo Laboratories for the thyroxine used. They also wish to acknowledge their indebtedness to Mr. D. S. Hart whose original observation led to the initiation of this work and with whom the month-by-month progress of the work was always discussed. Thanks are also due to Mr. J. Simpson of the Wool Department of the College for fleece gradings.

REFERENCES

COOP, I. E. 1953: *J. agric. Sci. 43:* 456.
FERGUSON, K. A.; CARTER, H. B.; HARDY, M. H. 1949: *Aust. J. sci. Res. B 2:* 42.
HART, D. S. 1954: *Nature, Lond. 174:* 1018.
——————1955: *Proc. N.Z. Soc. Anim. Prod. 15:* 57.
——————1957: *N.Z. J. Sci. Tech. A38:* 871.
HENNEMAN, H. A.; REINEKE, E. P.; GRIFFIN, S. A. 1955: *J. Anim. Sci. 14:* 419.
LABBAN, F. M. 1957: *J. agric. Sci. 49:* 26.

STUDIES IN HOGGET REARING

II: THE ROLE OF PARASITES IN HOGGET ILL-THRIFT

By E. A. CLARKE and DAISY B. FILMER, Ruakura Animal Research Station, Hamilton

(*Received for publication, 15 January 1958*)

Summary

Trials to determine the role of nematode parasites in the hogget ill-thrift syndrome were carried out over the years 1952-55 inclusive.

Comparisons of systems of rotational grazing and set-stocking showed that rotational grazing gave no advantage over set-stocking in the level of parasitism or the thrift of the animal.

Regular and frequent drenching with phenothiazine either under set-stocked or rotational-grazing management effectively reduced the level of parasitism but did not produce any worthwhile growth response in hoggets showing ill-thrift over the autumn and early winter.

Drenching gave most effective control of *H. contortus* and was less effective against *Trichostrongylus* spp.

Fine-particle phenothiazine (particle less than 10μ) was little more effective than coarse-particle phenothiazine in the control of *H. contortus* but showed a striking increase in the control of *Trichostrongylus* spp. in the small intestine.

The regular and frequent use of phenothiazine was shown to have no ill effects on the thrift of hoggets.

Hoggets reared on typical dairy-farm pasture remained thrifty throughout the autumn irrespective of parasite levels.

Hoggets reared on beef-fattening pastures, which were more akin to dairy-farm than sheep-farm pastures in length, showed no ill-thrift in the autumn in spite of varying levels of parasitism.

Hoggets reared worm-free and weaned on to worm-free pastures showed typical ill-thrift in the autumn on the fresh growth of grass which followed topping and autumn rain. A small secondary effect of parasites was demonstrated following oral administration of nematode larvae.

The interrelationship between plane of nutrition and level of parasitism is discussed.

It is suggested that the concepts of subclinical, clinical, and pathological levels of nematode parasites have been established with little reference to other factors of a more primary nature in the autumn ill-thrift syndrome.

It is concluded that nematode parasites with the possible exception of *H. contortus* play only a secondary role in the ill-thrift syndrome.

INTRODUCTION

In the first paper of this series (Clarke and Filmer 1958) mention was made of a trial by Roberts (1933) in which it was concluded that a condition of lambs in the autumn on old pasture, often said to be due to their grazing "sheep-sick" land, might be due either to parasitism or to some nutritional defect of the pasture. This question has remained an open one since that time.

Ewer (1946) from survey data and as the result of fattening trials with lambs in Canterbury, New Zealand, states that while deaths from enterotoxæmia are common "other deaths may follow an apparently acute sickness associated with scouring and rapid loss of condition, with the lamb dying within a few days; or a more gradually increasing unthriftiness may settle on many lambs, which will, in addition, show such symptoms as loss of appetite, thirst, persistent diarrhoea, with death eventually coming to a high proportion". In surveying the mortality among sheep in Canterbury in 1937-38, Ewer concluded that unknown factors associated with the feed itself were playing a part, but over the six years (1941-46) covered by trials this hypothesis remained unconfirmed. Ewer and Sinclair (1951) in reporting the results of these trials concluded that *Trichostrongylus* species are found in dangerously high numbers when climatic conditions favour worm multiplication. They stated *inter alia,* "It is then that the well-known clinical picture associated with severe worm infestation is seen; depression, and, in most instances, a persistent diarrhoea and a desire for water." Other considerations have led to the widespread belief that nematode parasites are indeed the prime cause of the autumn and early winter ill-thrift seen in hoggets. These may be briefly enumerated as follows.

1. Parasitological investigations over many years have shown the pathological nature of these nematode parasites. It has been demonstrated that subclinical burdens may have a depressing effect on appetite and growth. Gordon (1950) and Spedding (1952) have summarised much of the literature bearing on this subject.
2. One of the most consistent findings in post-mortems on unthrifty hoggets is a heavy parasite burden. Where heavy burdens of parasites are not found, the explanation is usually given that drenching had recently taken place, or that the animal had reduced its burden through the phenomenon of self cure or development of resistance (Gordon 1950; Stewart 1955).
3. The ill-thrift occurs from late summer onwards and in general would seem to be worst following moist humid conditions. These climatic factors are the most favourable to the propagation of nematode parasites. Furthermore, as the winter advances and conditions less favourable to the survival of parasite larvae develop, the ill-thrift condition tends to disappear and frequently clears up spontaneously.
4. Ill-thrift is confined to young animals which are known to be more susceptible to parasitism pending development of resistance.
5. It has been commonly observed that on new pastures, on dairy farm pastures, and frequently on crops, hoggets do well compared with their performance on old pastures. Again the assumption has been that old pastures are more heavily contaminated with parasites. Light rates of stocking likewise generally give good results. This has been presumed to be due to a reduction in intensity of exposure to the parasite larvae.

6. In unthrifty animals which are not subject to post-mortem examination, scouring, anaemia, and loss of condition are frequently seen.

These are commonly accepted symptoms of parasitism.

Furthermore, work in other countries, particularly Australia, has tended to high-light the highly pathogenic role of parasites and this outlook has tended to influence our assessment of the parasite problem. Past work in New Zealand, however, has shown that the parasite problem here is almost entirely confined to the hogget in the six months following weaning. There are exceptions to this, and occasionally pregnant or lactating ewes in some seasons and in some districts have been found to be heavily parasitised with *Haemonchus contortus.* In the autumn, ewes that have been overcrowded on pasture as a precaution against facial eczema have sometimes developed haemonchosis. Very occasionally lambs, before weaning, have been found with clinical symptoms of parasitism. Although sub-clinical levels of infestation could be of some importance in New Zealand in sheep other than hoggets, there is no evidence that this is so. In New Zealand, also, parasitism has a much more restricted seasonal incidence than in Australia (Tetley 1941, 1949; Whitten 1954) and fewer species have been shown to reach pathogenic levels.

Haemonchus contortus is clearly the most pathogenic species encountered in this country and is found throughout the North Island, but appears less widespread in the South Island. Ewer and Sinclair (1951) report its complete absence over the six years of their trials. Outbreaks of haemonchosis in hoggets resulting in serious losses are not uncommon. The epidemiology and symptoms of the disease conform to reports from other countries and are fully described by Gordon (1948, 1950).

Much work has been done that shows clearly that this parasite is effectively eliminated by anthelmintics, particularly phenothiazine. Gordon (1950) summarised much of the work done in this field and more recent work has fully confirmed these earlier findings. Because of its characteristic symptoms, comparatively easy recognition, known epidemiology in New Zealand, and ready elimination by anthelmintics, it cannot be regarded as important in the aetiology of the widespread ill-thrift of hoggets in the autumn and early winter, which persists sometimes for 5 months in spite of measures to control parasitism. It could not be dismissed entirely, however, as a factor in hogget ill-thrift because of its possible effects at varying sub-clinical levels, where there is an absence of striking symptoms. Furthermore, because of the high biotic potential of the parasite and the failure of the animal to develop resistance, re-infestation following control measures could possibly be of great importance.

It is clear from evidence in other countries and in New Zealand, and further confirmed by our experience over the five years of these trials, that haemonchosis can rapidly bring about severe anaemia, loss of condition, and deaths, or can be so rapid in its onset that deaths can occur before any marked loss of condition has occurred. Furthermore sheep can succumb irrespective of body condition, although there is evidence (Gordon 1950) that good nutrition enables an animal to withstand quite high levels of *H. contortus.* This parasite is to be regarded as producing a characteristic ill-thrift, and under New Zealand conditions can be regarded as a primary parasite, i.e. one capable of producing severe ill-thrift and mortality irrespective of condition and in some circumstances in spite of high nutritional levels.

The position of other nematode parasites in New Zealand is not so clear-cut. *Trichostrongylus* spp. are widespread throughout New Zealand and are frequently held to be responsible for lack of condition and for mortality in hoggets. Trichostrongylosis is insidious in its onset and in its early stages is readily confused with unthriftiness due to malnutrition. Its effects are long lasting and it may be several months before the animal recovers from a severe infestation (Gordon 1950). Its early symptoms are not striking and the parasite may be easily overlooked on casual post mortem. It is not very susceptible to anthelmintics including phenothiazine, particularly in its immature forms, although large doses give better control (Gordon 1950). More recently fine-particle phenothiazine has proved highly efficient.

Bennetts (1933) has reported that sheep suffering from trichostrongylosis die of malnutrition through loss of appetite. Andrews (1939) found a reduced intake of feed by infected sheep. Severe emaciation associated with malnutrition was noted only in sheep that survived for a relatively long period. Light infestations may not cause diarrhoea but heavy infestations are reported as causing severe scouring. Severe scouring, one supposes, would result in the desire for water -a symptom of ill-thrift reported by Ewer and Sinclair (1951). Franklin *et al.* (1946) found with moderate infestations a slower weight increase, depression in protein digestibility, poorer utilisation of calcium and phosphorus, and a reduction in wool production. Gordon says: "It appears that trichostrongylosis appears as a mild form of malnutrition, resulting from reduced appetite and reduced digestibility of protein, calcium, and phosphorus. The appetite continues to decline, and when diarrhoea supervenes the animal is virtually starved to death, because the small amount of food ingested passes through the alimentary canal too fast for digestive processes to be completed. If a low or falling plane of nutrition is concurrent, the development of trichostrongylosis is considerably accelerated." It is essentially a disease of young sheep, older animals apparently being resistant as a result of age and previous infestation. Under conditions of poor nutrition hoggets are more susceptible (Gordon 1950).

These and the further observations that the larval stages are relatively resistant to cold and desiccation resulting in a prolonged season embracing late summer, autumn, and early winter, over which animals could acquire infection, strongly suggested an association between the symptoms of trichostrongylosis and of hogget ill-thrift in New Zealand. Ewer and Sinclair (1951) found high numbers of *Trichostrongylus* spp. in unthrifty and dead lambs in Canterbury. In our trials and in survey material very high burdens of *Trichostrongylus* spp. have commonly but not invariably been found. A striking characteristic of the widespread ill-thrift in hoggets in New Zealand is that it so frequently occurs in a most severe form under conditions of feed supply both quantitatively and qualitatively good in terms of present-day knowledge. If indeed this ill-thrift be trichostrongylosis it is in striking contrast to its occurrence in other countries where obvious malnutrition is the most common predisposing factor.

There is little evidence of the pathogenicity of other nematode parasites in New Zealand. *Ostertagia circumcincta* has been reported in large numbers in lambs in the Marlborough district during one year and was suspected of causing an unthriftiness; Ewer and Sinclair (1951) found this to be the most common nematode in sheep in their trials but considered *Trichostrongylus* spp. to be the most pathogenic. In our trials it has been found mainly in the late summer. Although the other nematode parasites of sheep in New Zealand were present, they did not reach high levels nor have they commonly been found at high levels in post mortems on hoggets collected from a wide area.

Considerations such as those discussed indicated the need for some clarification of the role of nematode parasites in the aetiology of hogget ill-thrift. Many drenching trials have been carried out in the past with widely varying results, which are difficult to explain, in the absence of information on the efficiency of the drenching in removing parasites, the species of nematodes involved, and the level of nutrition of the affected sheep.

Whitten (1954) has reviewed published and unpublished data on some 60 drenching trials with phenothiazine involving approximately 5000 animals in New Zealand. In only two trials was a live-weight response of over 10 lb demonstrated and in 7 trials a response of 5–10 lb. In all other trials the body weight response was less than 5 lb or nil. In one experiment carried out under conditions of severe ill-thrift, however, drenching resulted in a marked reduction of mortality over the autumn and winter months.

EXPERIMENTAL

In an endeavour to clarify the role of parasites in the aetiology of hogget ill-thrift a series of trials was laid down over the seasons 1952-55 inclusive. In these trials, management was designed so that there was no shortage of food at any time. The aim was to see if hogget ill-thrift could be avoided or reduced by grazing management techniques or the use of anthelmintics aimed at keeping nematode parasitism at low levels. It is to be emphasised that in these trials practical drenching programmes were not under test. Phenothiazine was used because of its high efficiency and drenching was carried out at very frequent intervals in an attempt to eliminate nematode parasitism as a factor in hogget ill-thrift.

In all trials hoggets were selected from the Romney ewe-hogget flock bred at the Ruakura Hill Station. From this unculled flock of hoggets numbering some 600–700, groups were selected each year on a restricted random basis on live weight. In each year, therefore, groups were initially comparable in all respects and within groups ranged considerably in live weight, condition, and age. This range was considered desirable because of the widely held belief that late-born lambs and small lambs constituted the "tail end" of the hoggets which became evident as unthriftiness progressed.

Initially data were recorded under the following headings:

1. Changes in live weight and body condition (degree of fatness subjectively classified into grades, 1 extremely fat—10 emaciated) were used as the main criteria in assessing unthriftiness. These data were recorded at intervals of 2 or 3 weeks as permitted by weather conditions.

2. Nematode burdens.

   (a) Egg counts were made on faeces collected at the weighings. The modified McMaster technique was used (Gordon and Whitlock 1939 and Whitlock unpublished), corrections being made for consistency of faeces. The eggs were not cultured for larval identification of species nor was it possible to undertake the identification of the eggs of certain species because of the number of sheep involved in these trials. The limitations of egg counts as an assessment of worm burdens as discussed by Gordon (1948 and 1950) were fully appreciated. However, it was considered that frequent faecal examinations on large numbers of animals would enable a useful assessment to be made of the parasite status of the experimental hoggets and that these would indicate where more detailed work was warranted. The presence of coccidia oocysts and of eggs of *Strongyloides papillosus* and *Nematodirus* spp. was recorded but not included in total egg counts. Eggs of *Trichuris* spp. were omitted from egg counts.

   (b) Initially it was considered sufficient for the purpose of these trials to identify species of nematodes present by post-mortem examinations of the experimental hoggets.

3. At each weighing, notes were made on the incidence of "dagginess" and "scouring" and visual examinations were made to detect the presence of anaemia.

4. General post mortems were made on all animals dying in the course of the trials.

Trial 1: Rotational Grazing Versus Set Stocking: Ruakura Research Station, 1952

Several workers have claimed that rotational grazing results in better production both in wool and live-weight increase. Clunies Ross *et al.* (1932 and 1937) reported unsatisfactory gains in body weight and wool production in hoggets in the autumn and winter on natural pastures in Tasmania in spite of attempted control of parasites by drenching. They showed that rotational-grazing systems were definitely beneficial on parasitic infestations, live-weight gains, and wool production as compared with continuous grazing. It was not determined how far the variation in degree of parasitic infestation was due to the effect of the spelling period on viability of larvae, the differences in the closeness of grazing under the two systems, or the better nutritional level of the sheep under rotational grazing.

It has been suggested that under a system of set stocking the life cycle of the parasite is uninterrupted compared with a system of rotational grazing where periods of spelling must affect survival and viability of infective larvae. Theoretically, a rotational-grazing system which aims at grazing a paddock for only 3 or 4 days would result in eggs passed failing to reach the infective stage before the stock move on. Also the longer the spelling periods between grazings the greater the number of larvae failing to survive. This survival should be greatly reduced by the use of cattle as a "disinfecting agent" to precede the sheep, since parasitic nematodes are generally host specific although temporary infestation may occur (Clunies Ross 1931; Roberts 1942; Roberts *et al.* 1954; Bremner 1955). The shortness of the grazing period and the length of the spelling period are limited by practical considerations of pasture management and carrying capacity. The provision of special grazing practices for hoggets, which constitute a small proportion of total stock carried, can be subject to wide variation.

Objects of Trial

The main object in this trial was to contrast the effects of set stocking and rotational grazing. Under rotational grazing the paddocks were grazed for no more than 4 days and were spelled from sheep for 2 to 3 weeks and controlled by cattle.

Plan of Trial

A uniform paddock of 12 acres at the Ruakura Research Station sheep farm was divided into six 1-acre plots for the rotationally grazed group and three 2-acre plots for the set-stocked group. The plots were randomised over the area.

The pasture was spelled for one month before the commencement of the trial and controlled by mature cattle throughout the trial at the rate of three beasts to each treatment.

Pasture management in each treatment aimed at keeping the sward at about 3 to 4 in. long, and cattle were managed to achieve this. On the rotational area the cattle followed about a week after the hoggets on each plot and were removed to allow sufficient time for pasture growth to reach the desired height before the next grazing by the hoggets. Cattle were assisted in the control of the pasture by the use of a mower to top seed heads which were then more readily consumed by the cattle. Good pasture management was achieved by these means and throughout the trial all plots presented a well controlled uniform appearance and carried an abundance of high-quality pasture, of ryegrass, white clover, browntop, Yorkshire fog, cocksfoot, and paspalum. At all times there was a surplus of feed for the hoggets.

A random group of 35 hoggets weaned and selected on 7 January was used in each treatment. In the set-stocked group these were

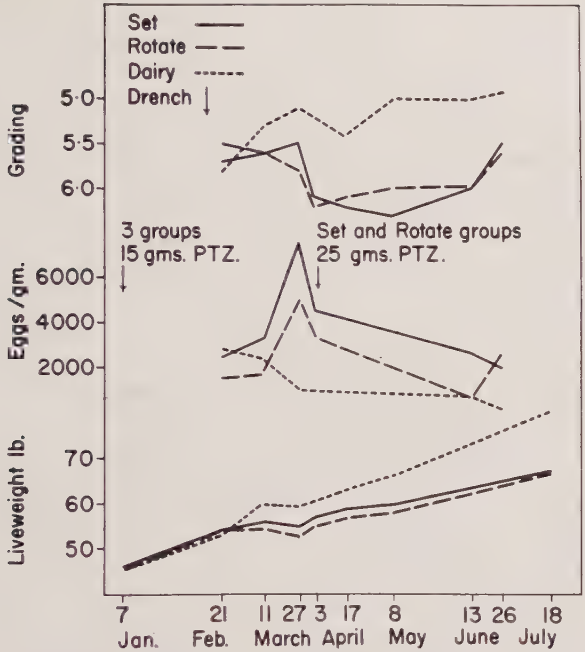


Fig. 1.—Trial 1: Changes in average live weight, grade, and worm egg count of hoggets set stocked, rotationally grazed and grazed on dairy-farm pastures.

divided between the three plots. This resulted in a stocking rate of approximately 6/acre. In the rotational group the hoggets were moved around the six 1-acre plots, grazing each plot for 3 or 4 days. All weights were recorded in the paddock.

Drenching with phenothiazine was carried out at the dose rates and on the dates shown in Fig. 1.

A group of 50 initially similar hoggets was run on a dairy farm at the Ruakura Research Station of similar soil type and carrying pasture of similar botanical composition, but under grazing management typical of a rotationally grazed dairy farm. Past work had indicated that the small groups of hoggets run on dairy farms grew well throughout the autumn and early winter. This group of hoggets provided a standard against which to compare the other two groups.

Results and Discussion

The live-weight curves of the three groups (Fig. 1) were the same until 21 February 1952, after which the dairy farm group showed a marked superiority. At no time were the mean live-weights of the rotated and set-stocked groups significantly different. The small difference shown between these two groups never exceeded 2 lb, and approximately 1 lb of this difference was due to the deaths of two animals which were below the mean weight (in the set-stocked group). It is interesting to note that all three groups suffered a check in live weight of similar intensity between the weighings made on 11 and 27

March. The dairy-farm group showed better recovery from this check. The variability of live weight within each group increased as the trial progressed. This increase was much less marked in the dairy farm group, which grew well. On 7 January, the standard deviations were 7.2, 7.3, and 7.7 for the rotated set-stocked, and dairy-farm groups respectively. On 25 July these had increased to 11.4, 15.2, and 8.9. This is in line with findings outlined in Part I where a marked individual variability within a flock in susceptibility to ill-thrift was noted. It was also found in these data that this variability was not related to the age or size of the hogget. The curves for mean body condition for the three groups change in accordance with and following live-weight changes in a fairly consistent manner. The behaviour of the dairy-farm group provides evidence that, in spite of frequent handling, experimental hoggets can grow at a very satisfactory rate.

The curves for mean egg counts showed differences between all three groups. In the rotational and set-stocked groups the curves were approximately parallel throughout, and the difference between these two groups was only of the order of 2000–2500 eggs per gramme (e/g) at any time. The level of parasitism in all groups was largely governed by the parasite burdens of a few individuals, e.g., the four animals with the highest counts in each group contribute more than

TABLE 1. WORM EGG COUNTS OF INDIVIDUAL HOGGETS CONTRIBUTING MOST TO THE GROUP TOTALS

Sheep No.	27/3/52	Sheep No.	12/6/52	Sheep No.	26/6/52
		ROTATE			
13	37,300	93	4,260	93	5,160
554	19,440	157	3,180	554	49,608
191	10,200	792	3,460	792	1,560
157	11,900	196	1,720	157	520
Total	78,840		12,620		56,800
Group Total	172,000		17,300		59,600
		SET			
179	22,800	179	13,720	179	25,320
200	50,190	200	15,000	200	13,440
513	81,120	800	15,320	78	1,790
399	18,780	193	10,800	800	4,240
Total	172,770		54,840		44,790
Group Total	257,500		68,300		48,937
		DAIRY			
767	6,406	132	5,400	913	800
718	3,800	218	1,950	40	800
231	2,730	690	1,950	35	689
222	2,600	118	2,200	27	520
153	3,400	27	2,000	118	520
3	2,730	32	1,400	218	520
Total	21,660		14,900		3,849
Group Total	52,980		31,110		6,973

half the total number of eggs per gramme for that group. When these four individuals were eliminated, the level of parasitism in the set-stocked group became very moderate. Inspection of the data reveals that at each sampling period it was not always the same few animals in each group that were influencing the mean level of parasitism. This is illustrated in Table 1.

Inspection of the data has shown also that the animals with the heaviest worm burdens have grown at a slower rate than the mean of the group but the correlation is not a particularly strong one (Fig. 2). It is interesting to record that at the time of the peak of infestation, i.e. 27 March, there was no correlation between weaning weight or age at weaning and the level of parasitism as assessed in eggs/gramme. This is illustrated in Figs. 3 and 4. It is clear that whether the difference in e/g between the set-stocked and rotational groups is significant or not, it is not reflected in the growth rates of these groups, which, compared with the dairy-farm group, grew equally poorly.

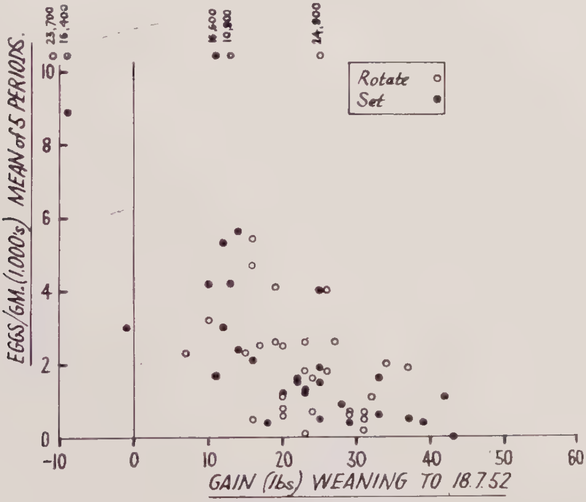


Fig. 2.—Trial 1: Relationship between worm egg count and live-weight gain in individual hoggets.

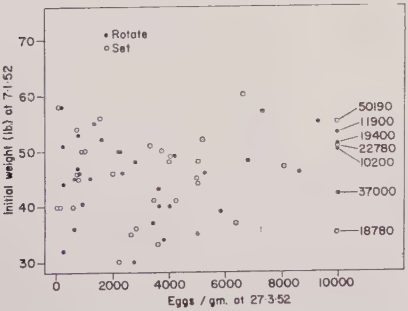


Fig. 3.—Trial 1: Relationship between weaning weight and worm egg count in individual hoggets.

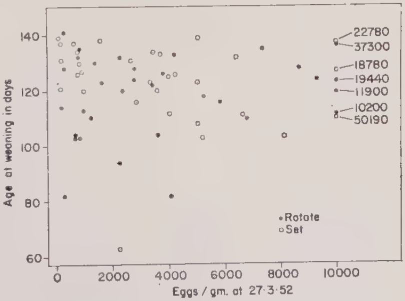


Fig. 4.—Trial 1: Relationship between age at weaning and worm egg count in individual hoggets.

The dairy-farm group had a lower mean egg count than the other groups, which might suggest a negative correlation between growth and parasite level. The low and declining level of parasitism in this group could be associated with the better growth rate or the lesser opportunity for reinfestation due to a much lighter rate of stocking. It is interesting to note however that all three groups showed some check in live weight and condition in late March, irrespective of egg count.

Whether the poor relationship between parasite level and changes in live weight is cause or effect cannot be clearly decided from these data.

Mortalities and Post Mortems

No deaths occurred in the dairy-farm and rotational groups, but four deaths occurred in the set-stocked group. The cumulative effect of these deaths lowered the group mean by less than 1 lb. Two of these died before the drenching in April. One of these animals had been an average lamb at weaning and had grown very poorly up to death. Its egg count never exceeded 2400 and on post mortem its worm burden was very low, there being only 20 *H. contortus* and a total of 6800 *Trichostrongylus* and *Ostertagia* spp. The other lamb that was below average at weaning lost weight and condition throughout the trial and its high egg count (up to 36,000) and the presence of anaemia suggested that it was haemonchosed. This was confirmed on post-mortem which revealed the presence of 5000 *H. contortus* and also 26,000 *Trichostrongylus* spp. Other species were present at moderate levels. The other two lambs died following drenching on 3 April. Both were below average in initial weight and growth rate. One only had a high egg-count (reaching 37,000 before drenching), progressive anaemia, and diarrhoea, which all suggested haemonchosis. Following drenching, anaemia and emaciation persisted, although at death on 5 June, *H. contortus* was not present though 27,000 *Trichostrongylus* spp. were found. The other lamb dying soon after drenching had low egg counts throughout, the maximum being 2600 e g, and at death had a low parasite burden with no *H. contortus* and 5000 *Trichostrongylus* spp.

Although all hoggets were drenched at weaning at the beginning of the trial and were immediately put on to the experimental plots which had been prepared by mature cattle and spelled from sheep for over a month, parasitism including haemonchosis rapidly developed. While drenching was less effective in removing *Trichostrongylus* spp. it is highly effective against *H. contortus* although immature forms may withstand drenching. The high biotic potential of this species is well-known and a few parasites surviving drenching and the pre-experimental pasture management would be responsible for the later development of haemonchosis.

Drenching on 3 April was carried out despite the fact that egg counts were falling at this time, probably owing to the self-cure phenomenon (Gordon 1950). At no time was excessive scouring noted in any group, but individuals in all groups, including the dairy farm, became "daggy." Only 50% of the animals in the two experimental treatments showed no "dagginess." The comparable figure for the dairy-farm group was 30%. This serves to illustrate the lack of association between parasitism and dagginess although within any group, animals which developed high egg counts showed diarrhoea and dagginess.

Summary and Conclusions

The main conclusions from this trial are summarised as follows:

The rotational grazing system showed no advantage over set-stocking on the growth rate of hoggets and both systems were much inferior to the system of lax grazing and light stocking on the dairy farm. All groups showed a similar check in growth in March.

Subjective estimates of body condition closely reflected live-weight changes.

Both the set-stocked and the rotated groups developed higher levels of parasitism assessed by egg counts than did the dairy-farm group and in all groups a few animals made a very large contribution to the mean egg count of the group.

Although the rotational grazing system used resulted in egg counts lower than those found in the animals under set-stocking, it failed to achieve the low level found in the dairy-farm group. A much better growth in the dairy-farm group was associated with much lower egg counts than in the other groups. At the higher level of parasitism shown in the set-stocked and rotational groups the small difference shown between the egg counts in these groups was not reflected in differential growth rates.

Over the period of the trial, a general negative relationship was found between mean worm burdens of individual hoggets (assessed by egg counts) and their live-weight gains. The correlation was not marked but in general the animals with high mean counts made less than average live-weight gains. It appears, however, that age and size at weaning were not correlated with level of parasitism as indicated by egg counts.

From these data it cannot be deduced whether the association between the level of parasitism due to a mixed infection of nematodes and the live-weight changes is cause or effect.

Trial 2: Rotational Grazing and Drenching:
Ruakura Research Station, 1953

Object of Trial

The object of this experiment was to see if a system of rotational grazing as employed in Trial 1, combined with regular and frequent drenching with phenothiazine, would (1) keep parasitism in hoggets at a low level; (2) give a growth rate approaching that achieved in similar hoggets on the dairy farm.

Plan of Trial

The random block used in the previous trial was further subdivided to provide 12 1-acre paddocks and six of these were allocated to the drenched group and six to the undrenched (control) group.

The preparation of the pastures and management throughout the trial was as in Trial 1. Grazing management consisted of 4 days' grazing and 20 days' spelling of each plot.

A random group of 30 ewe hoggets was selected at weaning and used in each treatment, i.e. drench and control. In addition, a similar group of 30 ewe hoggets was run on the dairy farm as in the previous year. Three wether hoggets were run with each group for killing to determine the species of nematode parasites present.

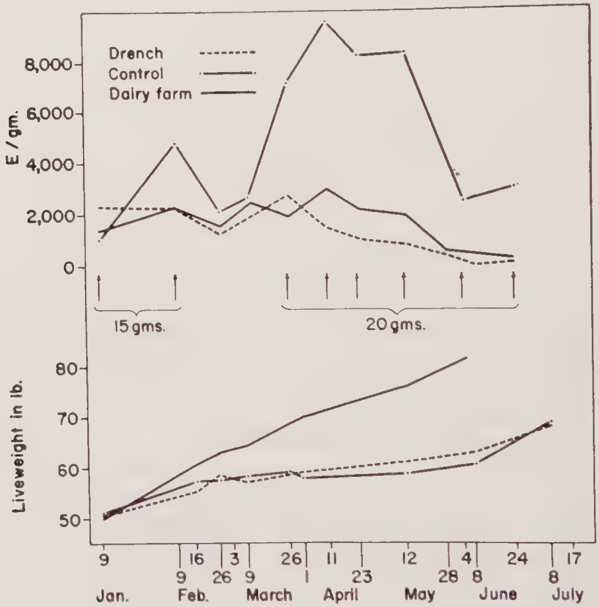


Fig. 5.—Trial 2: Changes in mean live weight and mean worm egg count in drenched and control hoggets, and hoggets grazed on dairy-farm pastures.

Drenching with phenothiazine was carried out at the dose rates and on the dates shown in Fig. 5. All hoggets were drenched at weaning on 9 January 1953. This was the only drench given to the control and dairy-farm groups. All hoggets were given 25 mg Calciferol on 3 March to eliminate the possibility of rickets complicating the results.

Results and Discussion

It is seen from Fig. 5 that as in Trial 1 the dairy-farm group was much superior in growth rate to the other groups which grew poorly and similarly throughout until June, when a marked improvement in the growth rate was shown in both groups. It is a striking characteristic of ill-thrift in hoggets that recovery in growth occurs spontaneously about mid winter without any change of paddock or treatment.

That the frequency of drenching may have depressed the growth of the drenched hoggets is not supported by the fact that both the drenched and undrenched groups grew similarly throughout and that both groups showed greatly improved live-weight from 8 June onwards although drenching continued until 24 June.

Subjective estimates of body condition showed similar trends to the live-weight curves.

Differences in worm burdens measured by egg counts between the control and the other two groups are clearly shown. The marked falls in egg count shown in the control group probably exemplify the self-cure phenomenon discussed by Gordon (1948), although the fall shown in May was probably not due to the more precise histamine reaction described by Stewart (1955) since a big intake of larvae at this time of the year is unlikely and was not indicated by subsequent egg counts.

Differences in egg counts between the drenched group and the dairy-farm group (which was not drenched) were at all times of small magnitude and suggest that the drenching programme was effective in reducing the parasite burdens to an even lower level than those of the dairy-farm group. As in Trial 1, the peaks of infestation shown in all groups are largely due to high counts in a few individuals. In the control group there are many more animals with counts greatly exceeding any recorded in the other groups.

Egg counts did not indicate the species of nematode present and it is known that an *H. contortus* infestation results in high egg counts whereas *Trichostrongylus* spp. are much less prolific egg layers (Gordon 1950). A sample of 16 wether hoggets from the Hill Station of the same origin and pre-weaning treatment as the lambs in this trial were killed on 22 January 1953. The numbers of nematode parasites present in these hoggets are shown in Table 2 and clearly indicate a mixed population with *Trichostrongylus* spp. in greatest numbers. Although *H. contortus* was present its numbers were not great. Table 3 shows the numbers of the different species present in hoggets post-mortemed throughout the trial. It is seen in Tables 2 and 3 that the nematodes of the large intestine and *Bunostomum* spp. were numerically of small importance. *Nematodirus* spp. and *Strongyloides* spp. were present also in relatively small numbers probably of no pathological significance. The eggs of these latter two species were not included in total egg counts.

Egg counts would be contributed to mainly by the worms of the abomasum and *Cooperia* spp. and *Trichostrongylus* spp. of the small intestine.

Gordon (1950) has emphasised the difference between the biotic potentials of the *Trichostrongylus* spp. and *H. contortus*. While a mature female *H. contortus* may produce 5000 10000 eggs a day, a female *Trichostrongylus* spp. may produce only 200 eggs a day. Based on the average numbers of these two species shown in Table 2 the daily output of eggs would be in the ratio of 7 *Trichostrongylus* to 12

TABLE 2. WORM COUNTS OF WETHER HOGGETS (UNDRENCHED) KILLED 22 JANUARY 1953 EX H.P. AND L.P. FLOCKS

H.P.	Mean Wt. (lb.) 14.1.53	Abomasum			Small Intestine					Large Intestine		
		Haem.	*Ost.*	*Trichs.*	*Trichs.*	*Coop.*	*Nem.**	*S.**	*Bun.*	*Trich.**	*Oes.*	*Chab.*
3	58	—	—	300	1,600	400	—	300	17	80	16	21
75	62	120	1,200	200	2,500	2,600	400	100	7	54	—	—
97	66	20	700	1,000	3,300	3,300	—	100	17	10	7	30
123	56	—	500	100	900	1,800	100	500	—	97	18	19
120	53	100	600	600	600	1,600	300	2,400	17	75	11	12
81	53	—	100	100	3,200	1,200	100	100	22	36	9	5
28	55		spilt		900	600	100	—	6	40	36	62
43	70	100	1,300	1,000	900	200	800	—	22	64	18	17
37	59	—	700	3,300	1,900	700	200	1,300	19	71	17	31
Mean	59.1	42.5	635	850	1,745	1,378	222	533	14	59	16	22
L.P.												
32	50	80	2,900	1,800	3,400	2,300	300	400	9	82	26	35
73	45	100	2,000	1,000	3,400	2,000	700	100	30	19	15	26
87	48	260	2,900	1,700	8,500	1,600	1,100	3,900	7	64	32	16
131	52	20	900	400	2,400	2,000	100	600	19	51	17	34
163	48	320	2,100	700	6,200	6,900	2,900	—	16	257	42	40
16	45	220	400	300	2,000	2,300	1,200	800	15	57	17	57
19	50	220	2,700	2,000	1,600	100	600	200	3	102	27	21
158	51	320	1,400	1,300	2,300	3,300	2,500	—	9	188	4	18
162	54		spilt		300	5,100	1,700	100	15	178	30	54
Mean	49.2	192.5	1,912.5	1,150	3,344	2,844	1,233	678	13	111	23	33

L.P.: Low Plane origin. H.P.: High plane origin. *Not counted in E/g. Oes: Oesophagostomum.

TABLE 3. PARASITE BURDENS IN INDIVIDUAL HOGGETS KILLED FROM DRENCHED, CONTROL AND DAIRY FARM GROUPS

Date	Stomach									Large Intestine								
	Haemon.			*Oster.*			*T. axei*			*Trichuris*			*Oesoph.*			*Chabertia*		
	Dy	Dr	C	Dy	Dr	C	Dy	Dr	C	Dy	Dr	C	Dy	Dr	C	Dy	Dr	C
9/3	1140	40	4360	2100	2000	2700	200	0	1300	9	98	6	31	87	30	28	38	18
1/4	540	40	11100	1000	700	4200	1200	100	3200	127	77	120	33	19	2	32	14	3
9/4*		0			100			0			43			7			0	
11/4			7460			3600			2600			139			30			75
13/5		0	0		0	100		200	8000		22	121		10	45		4	109
19/5	11			600			3700			2			0			3		
22/5			2560			6100			7300			67			2			0
2/5			11200			3300			4800			137			99			33

Date	Small Intestine														
	Trichs.			*Cooperia*			*Nematodirus*			*Bunos.*			*Strong.*		
	Dy	Dr	C	Dy	Dr	C	Dy	Dr	C	Dy	Dr	C	Dy	Dr	C
9/3	2900	4000	1700	2200	7100	200	160	900	180	8	0	0	900	2200	1800
1/4	4100	1200	6400	2400	7200	700	1700	1000	1200	1	8	0	0	1100	0
9/4*		5500			3500			0			4			800	
11/4			13100			5800			100			141			400
13/5		3400	13000		900	2100		0	0		0	19		300	3700
19/5	8300			11900			0			20			0		
22/5			14500			2100			300			20			700
2/5			—			—			—			—			—

* Found dead.
Dy = Dairy Group.
Dr = Drench Group.
C = Control Group.

Haemonchus.

It is seen from Table 3 that drenching has had its greatest effect in removing the worms of the abomasum, particularly *H. contortus*, *T. axei,* and to a lesser degree *Ostertagia* spp. These data indicate that lowered egg counts as a result of drenching were largely due to the removal of relatively few *H. contortus*, a highly pathogenic species. In the drenched group the egg count was contributed to mainly by the small intestinal *Trichostrongylus* spp. and *Cooperia* spp. and to a lesser extent by *Ostertagia* spp. In the dairy-farm group *H. contortus* was probably making a larger contribution to egg-counts than in the drenched group.

Although there are small differences in the population of worms in the dairy-farm and drenched groups, from what is known of the pathogenicity of the species present and the general level of the burdens carried it would seem most unlikely that the marked difference in the growth curves of these two groups could be attributed in any way to nematode parasites. It is also remarkable that in the drenched and control groups which did differ very markedly in parasite burdens the growth curves are identical.

Only one death occurred in the drenched group but parasitism was clearly not the cause—see Table 3.

As in the previous trial, anaemia was noted only in the control group in animals with very high egg counts. Diarrhoea was not noted in any group but there was a significantly higher incidence of dagginess in the control group than in the other groups.

Summary and Conclusions

Hogget ill-thrift was experienced in both groups of hoggets run on the sheep farm but not in the group run on the dairy farm.

It is clear that the rotational-grazing pattern followed was again quite ineffective in controlling parasitism in the control (undrenched) group.

The regular use of phenothiazine had a marked effect on the level of parasitism in the drenched group but did not increase its live-weight gains.

Hoggets on the dairy farm grew well and remained thrifty throughout in spite of a level of parasitism similar to that of the drenched group.

The data suggest that parasitism was not the cause of ill-thrift in these hoggets but was of secondary importance only.

Trial 3: Set Stocking Plus Drenching: Ruakura Hill Station, 1953

When drenched and undrenched animals are run together in the same paddock it has been argued that the full benefit from drenching cannot be achieved, since the undrenched animals are continually seeding the pasture with the eggs of the parasites they carry. The random block design in Trials 1 and 2 aimed at avoiding this possible complication. On the hill station the contour of paddocks did not permit a random block design.

Object of Trial

The object of this trial was to see if a system of set-stocking on hill country combined with regular and frequent drenching with phenothiazine to keep parasitism in hoggets at a low level would give a growth rate in hoggets approaching that achieved in strictly comparable animals run on the dairy farm (Trial 2).

Plan of Trial

The paddock used for this trial had been spelled from sheep for one month and controlled by cattle prior to the trial. After the first month of the trial the hoggets were moved to a paddock similarly prepared where they remained until June.

Two random groups of 30 ewe hoggets were selected at weaning and run together throughout.

One group was drenched with phenothiazine at the doses and on the dates shown in Fig. 6.

Results and Discussion

It will be seen from Fig. 6 that both groups grew equally well for the first six weeks and poorly over the next three months. Both groups showed a recovery soon after the middle of May. Differences in live-weight changes between the groups were of a very small order. By May the drenched group showed a superiority of only 2 lb over the undrenched group. It is interesting to note that over the first six weeks of this trial the live-weight increase in these hoggets closely approximated that of strictly comparable hoggets run on the dairy farm (Trial 2). This indicates that the quantity and quality of the feed in this trial over this period was adequate and no deterioration could be observed as the trial progressed until June when the quality of feed markedly declined.

As in Trial 2, it is shown that drenching has reduced the level of parasitism very efficiently as estimated by egg counts but has conferred little superiority to the drenched group in live-weight increase. Judging by the high egg counts and the presence of anaemia in a few individuals it is believed that a mixed infection similar to that shown in Table 2 was present in these hoggets initially. There were no mortalities. Dagginess was more marked in the control than in the drenched group.

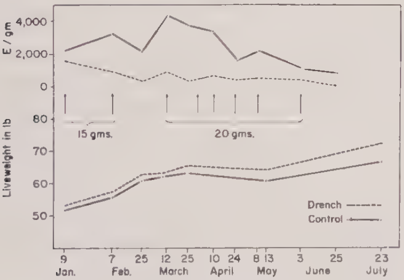


Fig. 6.—Trial 3: Changes in mean live weight and mean worm egg count in drenched and control hoggets.

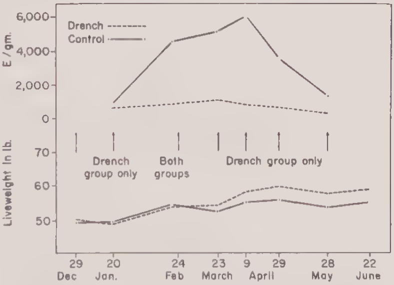


Fig. 7.—Trial 3: Changes in mean live weight and mean worm egg count in drenched and control hoggets.

This trial was repeated in the following year with very similar results as shown in Fig. 7. In this year recovery was slow, but this is accounted for on the grounds that the feed was totally inadequate from the end of June onwards. Over the main period of the trial from December to June inclusive there was ample feed at all times for these hoggets. The only deaths in this trial were due to pulpy kidney. One lamb from the undrenched group was killed on 22 April 1954 when it showed 6800 *H. contortus* and 13,400 *Trichostrongylus* spp.

Summary and Conclusions

The results of these trials support the conclusion arrived at in Trial 2 that ill-thrift was not due to nematode parasitism. Regular and frequent drenching markedly reduced the level of parasitism as judged by egg counts although drenched and undrenched animals were run together. In view of the frequency of drenching this was anticipated. As in Trial 2, all hoggets recovered in growth rate in winter in the same paddocks in which they had previously failed to thrive, an effect certainly not due to greater feed being available since grass growth had slowed down noticeably at the time.

Trial 4: Rotational Grazing Plus Drenching: Ruakura Research Station, 1955

The previous trials had indicated the need for more precise information on the nature of the parasite burdens of drenched and undrenched animals which showed ill-thrift over the autumn and early winter. It could be argued from the results of these trials that drenching, although highly efficient in removing *H. contortus*, was relatively inefficient not only in removing the small intestinal *Trichostrongylus* spp., known to be pathogenic, but also *Cooperia* spp. and *Ostertagia* spp. whose pathogenicity is not clearly known. Furthermore it was known that drenching was inefficient against the immature non-egg-laying forms of parasites whose pathogenicity was also not clearly understood. From the results of previous trials it was clear that egg counts alone presented an unsatisfactory picture of the effects of drenching, and furthermore Gordon (1950) reported that phenothiazine could depress the egg output of worms not removed by drenching. In this trial it was considered desirable to prevent *H. contortus* from dominating the picture, since its pathogenicity was so marked and well known. It was therefore decided that the control group would be drenched if individuals showed signs of haemonchosis as evidenced by very high egg counts and anaemia.

Object of Trial

The object of this trial was to gain more precise information on the effects of drenching on the parasite burdens of hoggets with particular reference to nematode species other than *H. contortus*.

Plan of Trial

The same area and the same random block system was used as for Trial 2. The preparation of the paddocks and the pasture and stock management was precisely as in Trial 2. Throughout this trial there was an abundance of well controlled feed. The rotational-grazing system was adhered to not because any superiority of this system over set stocking had been demonstrated but because

(1) rotational grazing reduced any possible paddock effects;

(2) it enabled very efficient pasture control and management to be carried out.

Wether hoggets were used. Each group consisted of 30 hoggets. In the drenched group 10 of these were used in a study of rumen flora and are not included in the results. This group of 10 hoggets ran with the drenched group throughout the trial and being similar hoggets in every respect served to adjust carrying capacity to the same number in each treatment.

Throughout the trial, five representative animals from each group were killed at the interval shown in the figures and worm counts made. In addition to mature forms a search was made for immature forms both in gut contents and the mucosa of the abomasa and small intestines. Mature females, and where possible the immature females, were identified for each species and recorded separately except in the case of *Trichuris* spp. which are numerically unimportant and do not contribute to the egg count, and *Strongyloides* spp. which are hermaphrodites. Very immature forms could not be sexed and were classified as one group called "immature mixed" for each species. All males identifiable as such were put into a separate category for each species.

Live weights and egg counts were determined and drenching carried out at the intervals and doses shown in Fig. 8A, B, C, and D. The control group was drenched once only on 13 April with coarse-particle phenothiazine when evidence of haemonchosis first became apparent. In this trial a sample of fine-grain phenothiazine (particle size under 10μ) was available and this was sufficient for the drenchings up to 13 April only, after which time coarse-particle phenothiazine was used.

Results and Discussion

The mean live weights and mean egg counts are shown in Fig. 8A-D. Fig. 8A shows the mean live weights and egg counts for the complete groups (drench group 20, control group 30) up to the first kill on 9 March. Fig. 8B shows the mean live weights and egg counts for sub-groups (drench group 15, control group 25) comprising those animals that were present throughout up to the second kill on 13 April and so on for Fig. 8C and 8D.

It will be seen from these figures that

(1) There was a striking similarity between the pattern of the comparable growth curves shown in the four figures and to a lesser extent this applies to the curves of mean egg counts.

(2) There was also a striking similarity between the pattern of growth curves of the drenched and undrenched sub-groups.

(3) Even where mean body weights between sub-groups differed initially owing to the selection of a range of animals within the groups for killing, these differences have been maintained except where egg counts became unduly high in March in the control sub-group (Fig. 8C and D). This confirms our findings reported in an earlier paper that the size of the hogget is not a factor governing the onset or severity of ill-thrift.

(4) Growth was good (approximately 3 lb/week) until the beginning of February in both the drenched and control animals and thereafter was most unsatisfactory for the next $3\frac{1}{2}$ months. The

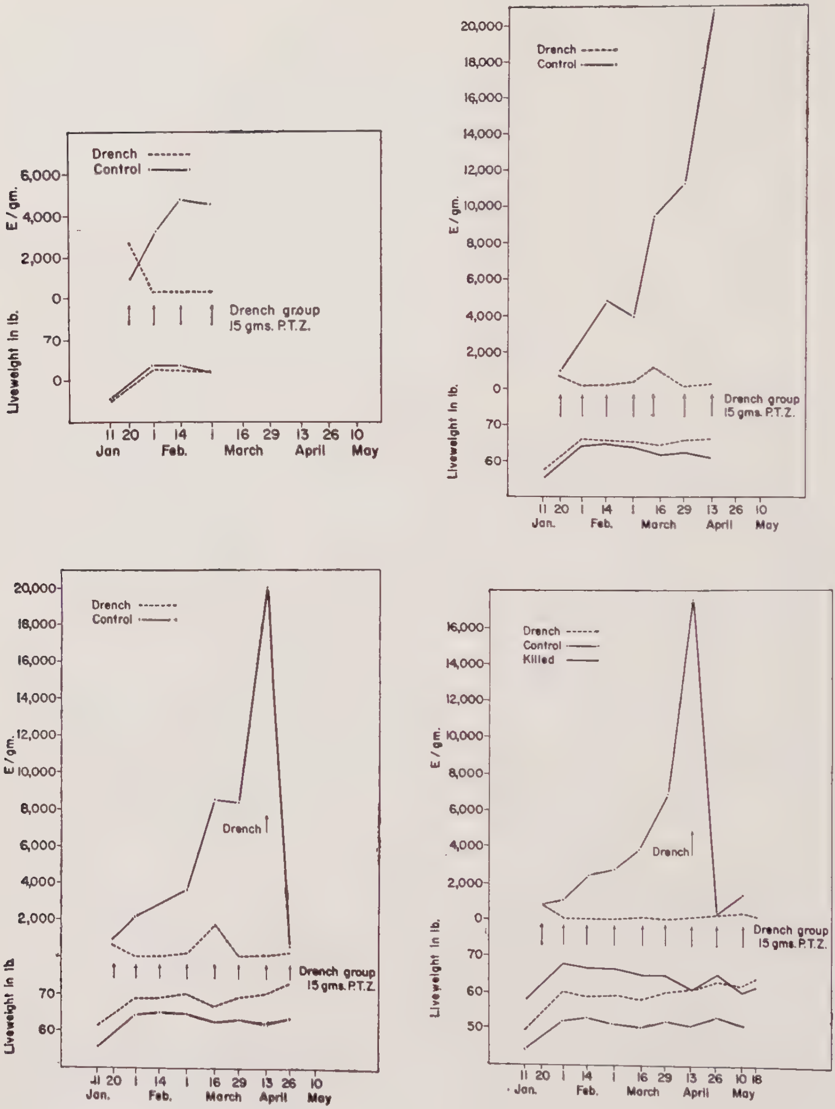


Fig. 8.—Trial 4: Changes in mean live weight and mean worm egg count in drenched and control hoggets: A (top left) initial group. B (top right) remainder after first kill. C (bottom left) remainder after second kill. D (bottom right) remainder after third kill.

improvement in growth shown in May (Fig. 8D) was maintained at a rate of approximately 1½ lb/week in the 10 animals remaining in the control group after the last kill on 18 May. This illustrates once again the spontaneous nature of recovery from ill-thrift.

(5) As in the previous trials, the onset of ill-thrift preceded the peak of parasitism.

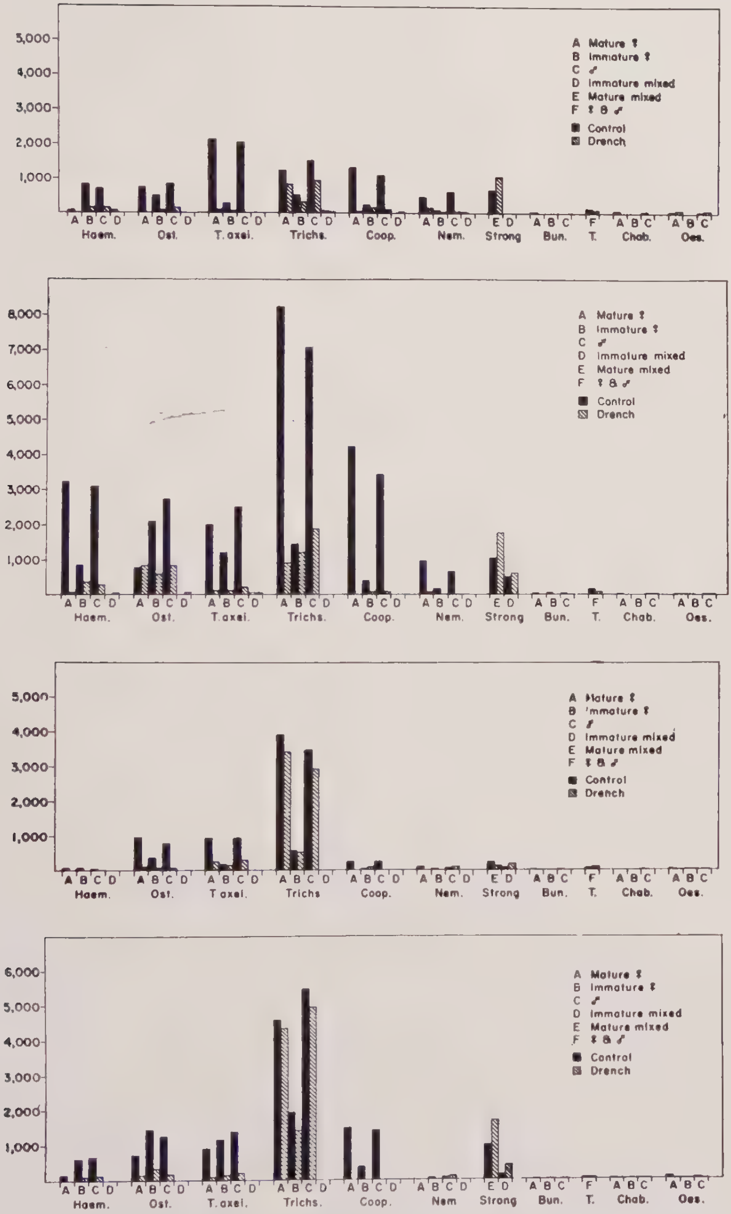


Fig. 9.—Trial 4: Numbers and species of parasites in hoggets from drenched and control groups. From top: A. Killed on 9 March; B. Killed on 13 April; C. Killed on 5 May; D. Killed on 19 May.

(6) In the drenched group, egg counts have been very low throughout the trial, but in spite of this the typical depression in growth curve is seen throughout the autumn and early winter. The small peak in mean counts shown for this group (Fig. 8B and C) on

16 March is due mainly to one animal with a count of 9800 e/g. This animal was possibly not drenched or faultily drenched on 1 March. On 29 March it had a negative count. The fall in mean live weight for the drenched group was seen in all animals in the group irrespective of their egg counts.

(7) The high parasite levels reached in the control group had very little differential effect on the growth curve and this only after ill-thrift had persisted for about 2 months.

The mean worm burdens of the animals killed from each group at the four periods are tabulated in Fig. 9A, B, C and D. It will be recalled that the eggs of *Nematodirus* spp., *Strongyloides* spp. and *Trichuris* spp. are not included in egg counts. Lungs were examined for nematodes and the only species found were *Dictyocaulus* spp. The greatest number recorded was 20 in one animal and in most cases they were absent or fewer than 5. Fig. 9A and B present the picture with respect to parasites in the drenched group and in the control group prior to the drenching on 13 April. At the time of the first kill on 9 March made some 8 days after drenching, the mean egg count was very low in the drenched group and was derived mainly from the mature females of *Trichostrongylus* spp. in the small intestine, there being very few mature females of other species present. In the control group, *Trichostrongylus* spp., *Ostertagia* spp. and *Cooperia* spp. contributed to a mean egg-count of approximately 500 e/g. There were only about 70 mature females of *H. contortus* present. It is interesting to note that both groups at this time had been unthrifty for over a month, over which time parasite burdens had been very much below pathogenic levels.

Drenching effectively reduced all species except *Strongyloides* spp. but was less effective against mature and immature forms of the intestinal *Trichostrongylus* spp. In the case of *H. contortus* mainly immature forms were present indicating the commencement of the build-up of these species which by 13 April or five weeks later had reached a high level averaging about 7000 worms (Fig. 9B).

At the time of the second kill on 13 April the mean egg count had reached a high level of 20,000 e/g in the control group and was almost negative in the drenched group. Fig. 9B shows that the low count in the drenched group was due mainly to mature female *Trichostrongylus* spp. in the intestine and *Ostertagia* spp. There were almost no mature female *H. contortus*. In the case of the control group, however, there were heavy burdens of mature females of *Trichostrongylus* spp., *Cooperia* spp., *H. contortus*, *T. axei* and *Ostertagia* spp. in that order.

In general, the high efficiency of fine-particle phenothiazine has been demonstrated. It has practically eliminated mature forms of *H. contortus* but there is a suggestion that it is less effective against immature forms. The extent however to which immature forms have been acquired since the last drenching 18 days previously cannot be estimated. It will be noted that *Ostertagia* spp. are present in greatest numbers in both groups at the time of the second kill. The explanation of this may be epidemiological.

At the time of the third kill on 5 May the mean egg count in each group was low, 400 e/g in the control group and 100 e/g in the drenched group. In the case of the control group, egg counts had been reduced by drenching on 13 April because haemonchosis was becoming evident. Thus the animals were killed 3 weeks after drenching in the case of the control group and 9 days after drenching in the drenched group. Coarse-particle phenothiazine was used and had been used for the drenched group on 13 April and thereafter.

In the drenched group *H. contortus* was absent. The only species remaining in any numbers are *Trichostrongylus* spp. in the intestine which accounted almost wholly for the egg count recorded. In the control group, worm burdens were slightly greater than in the drenched group and egg counts are contributed to by *Trichostrongylus* spp. and *Ostertagia* spp. The low efficiency of coarsely ground phenothiazine at the dose rates used against mature and immature *Trichostrongylus* spp. is clearly seen.

At the time of the fourth kill on 19 May the mean egg count of the drenched group was only 225 e/g and of the control group 1400 e/g. In the control group there was a period of 5 weeks and in the drenched group 9 days since drenching. The egg count in the undrenched group was mainly due to *Trichostrongylus* spp., *Ostertagia* spp., *Cooperia* spp. and to *H. contortus*. In the drenched group, the egg count was mainly due to intestinal *Trichostrongylus* spp. The inefficiency of coarsely ground phenothiazine at the dose rate used against immature and mature *Trichostrongylus* spp. of the intestine is again confirmed. It would seem that better control of intestinal *Trichostrongylus* spp. is achieved when they are present in greater numbers.

Growth in both groups was poor over a period of 4 months, during which time parasite burdens varied considerably in the numbers and species present within and between the two groups. The evidence suggests strikingly that parasites played no part either in the onset or the duration of the ill-thrift, though the divergence of the growth curves from the end of March onwards suggests that they played a small secondary role. These points will be amplified further in discussing Trial 5 where the worm burdens of thrifty hoggets initially comparable in every respect to those used in this trial are presented.

The evidence from this and past trials suggests that drenching at weaning is most likely to be unwarranted. This may not be true in an exceptional year where wet moist conditions occur early in the season favouring an earlier build up of *H. contortus* in particular.

Summary and Conclusions

Drenching as used in this trial kept parasites in the drenched group at a very low level in comparison with the control group. Both mature and immature forms of *H. contortus* in particular were practically eliminated by drenching. In the early stages of the trial, fine-particle phenothiazine greatly reduced the number of mature and immature *Trichostrongylus* spp., but in the later stages of the trial coarsely ground phenothiazine proved very inefficient.

Ill-thrift developed at the same time and was of the same intensity and duration in both groups throughout the trial and persisted for about four months. This was in spite of very marked differences between the levels of parasitism in the two groups.

Furthermore, ill-thrift developed while parasite levels were low in both groups.

The evidence suggests strikingly that mature or immature nematode parasites played no part either in the onset or duration of ill-thrift. The divergence of the growth curves of the two groups at the end of March after ill-thrift had been present in both groups for 2 months suggests that the parasites concerned played a small secondary role in this ill-thrift of hoggets.

Trial 5: Hogget Rearing on Beef-fattening Pastures: 1955

Object of Trial

The object of this trial was to determine whether thrifty hoggets could be reared through the autumn on pasture normally grazed by beef cattle; and whether such hoggets infected with heavy burdens of nematode parasites of mixed species with a low *H. contortus* level would also thrive over the autumn.

Plan of Trial

Three groups of 10 wether hoggets identical with those used in Trial 4 were used in this trial. All hoggets were drenched 3 times with 15 g phenothiazine prior to the commencement of the trial on 3 March 1955.

Group 1 (Infected). The animals in this group were given *per os* mixed infections of nematode larvae on 3 March and pure *Trichostrongylus* spp. thereafter at the levels and on the dates shown in Fig. 10A and 10B.

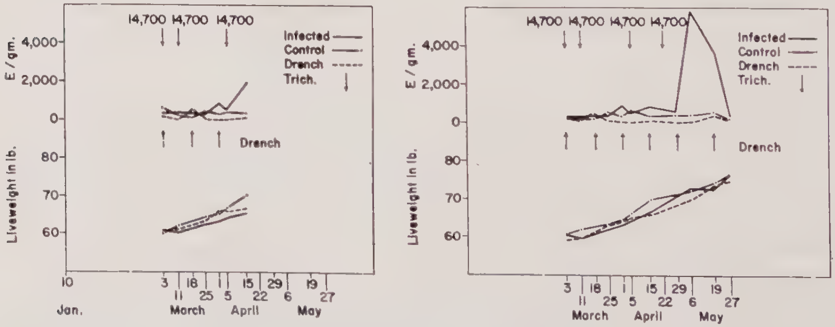


Fig. 10.—Trial 5: Changes in mean live weight and mean worm egg count in infected, drenched, and control groups. Left. A. Initial groups; Right. B. Animals killed on 1 June.

Group 2 (Control). The animals in this group were not artificially infected. They were not drenched during the trial.

Group 3 (*Drenched*). These were drenched with 15 g of fine-particle phenothiazine until 1 April on the dates shown in Fig. 10A and B. Thereafter ordinary commercial phenothiazine was used.

Three paddocks of approximately 10 acres were used, which prior to the trial had been well controlled by mature beef steers. No sheep had grazed the area for several years. The pasture was very similar to that used in the Ruakura trials but carried more ryegrass and much less paspalum.

One paddock was allotted at random to each group. However, by April it was believed that a paddock effect might be responsible for the better growth of the control group and thereafter at weekly intervals the groups were shifted in an orderly rotation. At no time was there any shortage of pasture which was more akin in length to a dairy-farm than to a sheep-farm pasture.

As in Trial 4, representatives from each group were killed and worm counts made on the dates shown in Fig. 10A and B.

Results and Discussion

Mean live weights and egg counts for all the animals in each group are shown in Fig. 10A. Fig. 10B gives these data for the animals killed at the end of the trial.

It will be seen that there was no significant difference in the live-weight gains of the three groups and that there was no ill-thrift. The small fluctuations shown are believed to be paddock effects. Only in the infected group did egg counts reach high levels. The marked fall in egg count of the infected group on 6 April was a spontaneous one similar to those discussed in previous trials. The failure of the control group to become heavily parasitised was undoubtedly contributed to by the light rate of stocking and possibly to the thrift of these animals.

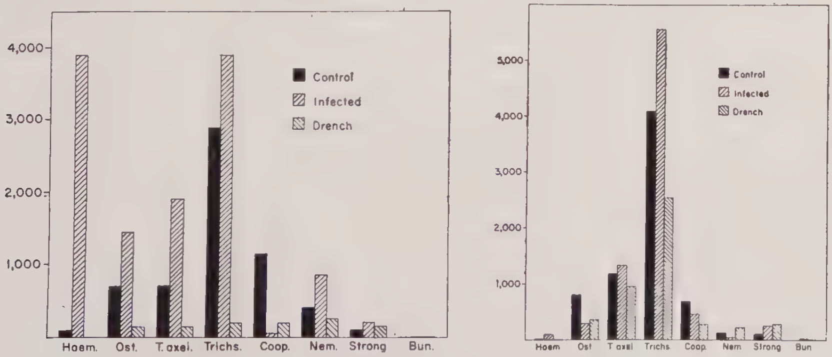


Fig. 11.—Trial 5: Mean numbers and species of parasites in two animals from each group. Left. A. Two hoggets killed on 15 April. Right. B. Eight hoggets killed on 1 June.

The worm counts for the two animals killed on 15 April and of the 8 animals killed on 1 June are shown in Fig. 11A and 11B respectively. In Fig. 11A, while the animals in the group drenched with fine-particle phenothiazine showed a low count for all species, there is evidence in the control group of a moderate level of *Trichostrongylus* spp. but very few *H. contortus*. In the infected group, however, there was a very heavy burden of *H. contortus* and a moderate burden of *Trichostrongylus* spp. The *H. contortus* burden was carried mainly by one lamb with a count of 7000 mostly immature worms. The animals killed at the conclusion of the trial (Fig. 11B) showed heavier levels of *Trichostrongylus* spp. in all groups, which points to the lower efficiency of coarse-particle phenothiazine. The high level of *Trichostrongylus* spp. in the infected group is due mainly to immature forms which explains the low egg count recorded a few days previously.

In this trial the different levels of parasitism in the three groups are not reflected in any way in the growth curves and suggest that sub-clinical levels of parasitism are of doubtful importance in the growth of hoggets.

The hoggets in this trial provide groups of thrifty animals with which to compare the unthrifty ones of Trial 4 as regards parasite burdens carried. This comparison is a valid one to the extent that both groups were of the same origin and of the same average weight and weight range at weaning, and received no marked differential treatment other than in the trials concerned. Whereas the mean egg count of the drenched group in Trial 4 never exceeded 2000 e/g throughout the prolonged ill-thrift period (Fig. 8), in the thrifty but infected group in Trial 5 the average count exceeded 5000 e/g (Fig. 10). A comparison of Figs. 9 and 11 shows that it was only after ill-thrift had been present in the drenched group at Ruakura for some 4 months that their worm burdens approximated and slightly exceeded those found in the infected group at the beef farm. For the first 3 months of the ill-thrift period in the Ruakura drench group the worm burdens were lower than those of the infected group on the beef farm. This is in striking support of the contention previously advanced that parasites were not the cause of ill-thrift in hoggets over the autumn.

Summary and Conclusions

Hoggets run at a light rate of stocking on a beef-fattening farm throughout the autumn and early winter remained thrifty on dominant white clover and ryegrass pastures.

A parasitised group attained a moderate degree of parasitism mainly of *Trichostrongylus* spp. but grew as well as drenched and control groups which were relatively free from parasites.

No effect could be seen in the growth curves of the groups which could be related to the different and changing levels of parasitism within and between groups. This suggested that effects on growth of sub-clinical levels of parasitism on hoggets under these conditions were of no importance.

The level of parasitism in the parasitised group in general was similar in nematode species concerned and for the majority of the trial heavier than that in a similar group of drenched hoggets failing to thrive on a sheep farm (Trial 4).

Trial 6: Ill-thrift of Parasite-free Lambs: Ruakura Research Station, 1955

Object of Trial

Earlier studies had suggested that nematode parasites were probably not the cause of ill-thrift in hoggets in the autumn. Evidence from trials to be presented in Part III of this series of papers suggested that pasture-management systems aimed at keeping the sward short and well controlled by the use of cattle and topping with the mower, under conditions of good pasture growth, were conducive to ill-thrift particularly under heavy rates of stocking. The freedom from ill-thrift on the dairy farm was believed to be largely if not entirely due to the more lax pasture management, resulting in longer feed and the lighter rates of stocking with hoggets. It was believed that sufficient was known of pasture management to produce ill-thrift in hoggets under conditions where nematode parasites were absent and thus demonstrate conclusively that parasites were not the cause.

Plan of Trial

Selection and Management of Animals (Pre-experimental period). Lambs were weaned at 3 days of age and kept worm-free by hand-feeding indoors in pens until approximately 3 months of age when they were put on worm-free pasture. The hand-rearing technique will be reported in a separate communication (Filmer and Clarke). Since *Strongyloides* spp. were considered to be of no importance in this trial no attempt was made to keep this parasite out of the picture.

Parasite-free Pastures. The provision of parasite-free pastures was not completely achieved. A paddock of approximately 8 acres at the Ruakura Research Station was the only area available which was likely to be parasite-free. This area was known to have been grazed only by bulls for over 12 years. Though likely to be free from sheep parasites it was likely to be infested with some cattle parasites which were expected to be unimportant. To test freedom from parasites two lambs born in July were reared worm-free and run on part of this area from October. Egg counts on faecal material made at intervals showed the presence of a few *H. contortus* eggs. These lambs were killed on 10 March when very small numbers of *H. contortus* were the only species of nematode found. These were probably of cattle origin (Roberts *et al.* 1954) although this was not confirmed. This was the most undesirable species to be present because of its high biotic potential and its well known ability to cause severe and characteristic ill-thrift in hoggets largely irrespective of their condition and nutritional level. Fortunately, it is most readily controlled by drenching and it was therefore decided that if drenched regularly the experimental lambs would for all practical purposes be free from parasites. That this was so is seen from Figs. 12 and 13.

The paddock was initially divided in half and the lambs grazed one of these blocks until they were allotted to experimental groups. The remaining block was hayed and subdivided into four 1-acre plots. Each plot was separated from neighbouring plots and paddocks by double fences enclosing a shallow drain, to reduce the risk of infestation with parasites. Precautions were taken against trespass, and workers before entering the worm-free area changed into clean boots kept for the purpose. Obviously it was impracticable to aim at perfect isolation.

Twenty-eight wether hoggets reared worm-free as above were randomised on a body-weight basis into two groups on 14 February after being shorn in the paddock.

Group 1 (Worm-free). With the aid of drenching throughout the trial almost complete freedom from worms was achieved.

Group 2 (Infected). This group was run to simulate a group of hoggets on pasture carrying the usual mixed infection of nematode parasites and to provide a control group. A mixed infection excluding *H. contortus* would have been desirable. This was not possible and the mixed culture used contained a few of this species. It was decided that *H. contortus* would be kept in check, if necessary, by small doses of phenothiazine, and for this reason this group was dosed with 10 g of phenothiazine on 10 May (Fig. 12). The hoggets were inoculated *per os* with a mixed infection of nematode larvae on 1 March and thereafter with *Trichostrongylus* spp. on the dates and with the numbers shown in Fig. 12.

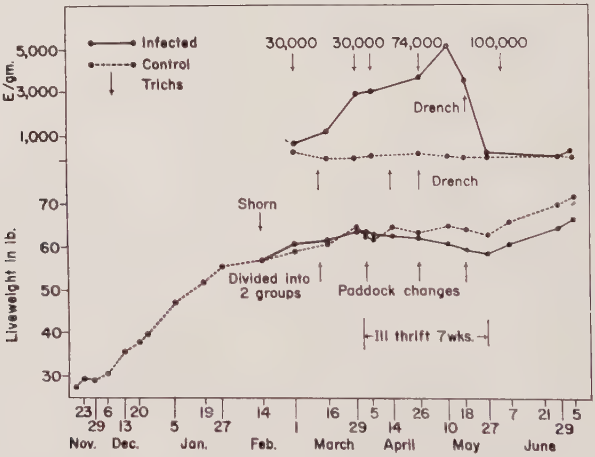


Fig. 12.—Trial 6: Changes in mean live weight and mean worm egg count in "parasite free" and infected hoggets.

Two of the one-acre plots were allotted at random to each of the experimental groups and each group was rotated between its two plots so that at no time was there a shortage of feed. Plot changes are recorded in Fig. 12. Management aimed at providing feed 3 to 4 in. long. To achieve this, the mower had to be used to control seed heads.

All animals were killed at the conclusion of the trial and worm counts were made.

Results and Discussion

The growth rate of the lambs while on pasture from 22 November is given in Fig. 12. Prior to the randomisation into the two experimental groups the live-weight increase per week averaged about 3 lb. Over this period the hoggets were running in a 4-acre paddock which had not been hayed and which was long and uncontrolled. Following randomisation on 14 February the hoggets grazed the experimental plots which had been prepared by haying and carried initially a well controlled sward somewhat more than 4 in. long. In spite of dry conditions the feed was not well controlled, although the rate of stocking was relatively high. Over the period ending 2 April, the feed had been long and more typical of a dairy than a sheep pasture. Just prior to the paddock change on 2 April, however, much tall pasture and seed head was evident and much less leaf was available. It was thought that the sudden drop in live weight about this time was due to the obvious deterioration in feed quality. The plots to which the hoggets were changed had been topped on 11 March and carried a fresh growth of pasture some 4 in. high. This was the first approximation to typical sheep pasture on which the hoggets had been grazed.

Occasional topping, and plot changes as necessary, kept the pastures in this condition thereafter. At all times there was an abundance of leafy feed 3 to 4 in. high and this continued until July when the trial ended. Feed was less plentiful towards the end of the trial when the hoggets were again growing well.

From 2 to 26 April the growth of the hoggets was most unsatisfactory in spite of an abundance of feed, and the growth curves of both groups were almost identical.

Egg counts were generally negative in the parasite-free group, but in the infected group built up to a peak of over 5000 e/g on 10 May. There is no evidence of an effect on the growth rate of the infected group from this parasite burden until the peak was reached when the mean live weight fell to about 5 lb below that of the parasite-free group. This difference was maintained thereafter. At the time of this peak, blood examination revealed the presence of occasional stippled red cells and somewhat low haemoglobin values in 6 hoggets in the infected group. The blood of all the parasite-free hoggets was normal in these respects. The infected group was therefore drenched with 10 g phenothiazine on 18 May. The recovery in growth rate in both groups

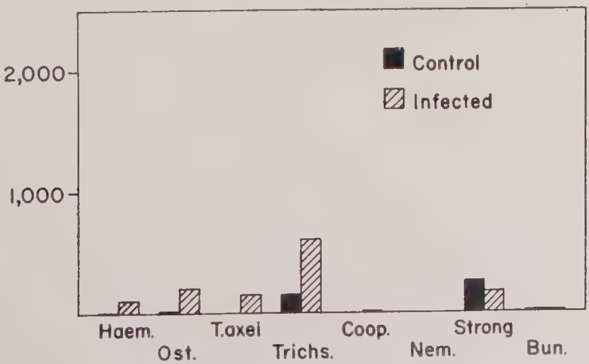


Fig. 13.—Trial 6: Mean numbers and species of parasites in infected and "parasite free" hoggets killed on 5 July.

from 27 May onwards is typical of the spontaneous recovery from ill-thrift which usually occurs about this time irrespective of pasture management.

Worm counts were made on all hoggets on 5 July and the mean values for each group are shown in Fig. 13. In the parasite-free group a few *Trichostrongylus* spp. of the intestine and *Strongyloides* spp. were present. It is clear from these findings and from the egg counts throughout the trial that almost complete freedom from parasites has been achieved. The number of parasites found in the infected group at post-mortem is low. This is in some degree due to the drenching of this group on 18 May and was undoubtedly higher at the peak of infestation. It would require a big increase in the number of parasites of every species present at post-mortem to account for the egg count recorded on 10 May. If the peak were due to *H. contortus* alone it would indicate the presence of something like 1000 worms of this species. In spite of an inoculation of 100,000 larvae of *Trichostrongylus* spp. on 3 June the level of parasitism did not build up again, suggesting that a resistance may have been established.

No obvious pathological abnormalities were found at post-mortem or throughout the trial except those due to slight haemonchosis in the infected group. No diarrhoea was observed in the hoggets but from 7 June onwards the faecal samples collected from both groups were recorded as soft and many in the infected group as very soft.

Summary and Conclusions

In this trial ill-thrift in hoggets in the presence of an abundance of autumn feed developed in the absence of parasites. This was believed to be due in part to the pasture management used.

The ill-thrift was no more severe in the parasitised animals until mild haemonchosis developed and was diagnosed from blood examination.

The ill-thrift was manifested only in rate of live-weight gain and no obvious pathological abnormalities were noted on post-mortem nor were they observed throughout the trial except in the infected group at the time mild haemonchosis developed when slight anaemia was diagnosed in a few hoggets.

Prior to the onset of ill-thrift the growth rate of all hoggets though slow was satisfactory on a pasture which became progressively long and stemmy.

The onset of ill-thrift coincided with the change to abundant short leafy pasture which grew rapidly following topping of the paddock and a heavy fall of rain.

As in past trials, ill-thrift spontaneously disappeared though no change in the pasture could be observed.

Trial 7: The Effect on Thrifty Hoggets of Regular Drenching with Phenothiazine

There is little evidence of toxic effects from the use of phenothiazine on sheep, and Gordon (1950) states that toxic effects are of no significance if one excepts ewes in the last stages of pregnancy. Kidney lesions associated with red water after drenching with phenothiazine were reported in the autumns of 1951 and 1952 and in the summer 1952-53 in unthrifty lambs (Salisbury *et al.* Pers. comm.).

Throughout the trials reported in this paper phenothiazine had been used at very frequent intervals in an endeavour to control parasites. No evidence of ill effects of the phenothiazine had been seen and, as already discussed, evidence in Trial 2 suggested strongly that phenothiazine did not affect the growth of hoggets.

In the 1954 season a group of thrifty hoggets on the dairy farm was available to provide further information on the effects of regular drenching with phenothiazine on growth rate.

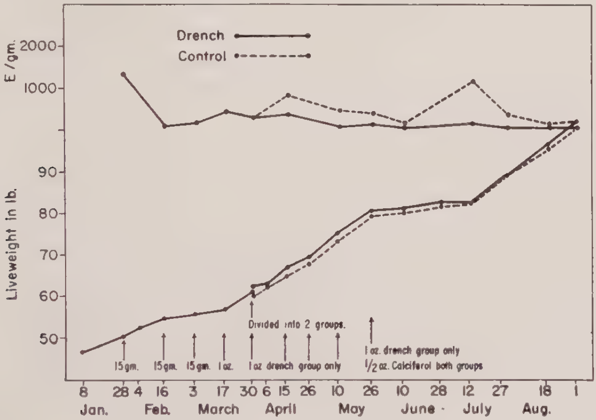


Fig. 14.—Trial 7: Mean live weight and mean worm egg count in frequently drenched and control hoggets.

This group had been grazing on the dairy farm from 8 January 1954 and had been drenched 4 times with 15 g phenothiazine before they were divided into 2 similar groups on 30 March (Fig. 14). From this date onwards one of these groups only was drenched with phenothiazine at the levels and on the dates shown in Fig. 14.

Initial selection resulted in a slight difference between mean live weights of the two groups and this was maintained throughout the trial. Both groups grew very well and after shearing on 2 October the mean weights were 98 lb and 97.3 lb for the drenched and the control groups respectively. There was no difference in fleece weights.

Level of Nutrition and Parasitism

Much work done on the association between the level of nutrition and resistance to parasites has been summarised by Culbertson (1941). Studies by Clunies Ross (1932), Clunies Ross and Gordon (1933), and Fraser and Robertson (1933) clearly indicate a negative relationship between level of nutrition and level of parasitism in the animal. Gordon (1948) shows the effect of a better ration in rapidly lowering an infestation of *H. contortus.* Another trial by Gordon and Johnstone (ibid.) has shown that nutritional levels may have a differential effect on different species of parasites. On high-level feeding on oats sheep threw off an infestation of *O. columbianum* but rapidly built up infections of *H. contortus* and *Trichostrongylus* spp. In spite of the burdens carried, the sheep on the higher plane of nutrition grew rapidly whereas comparable sheep on a low level of nutrition on pasture lost weight and many died.

We have some data which suggests that a high level of nutrition enables an animal to grow well in spite of quite heavy parasite burdens. The excellent growth rate and freedom from ill-thrift of hoggets on white clover in the Gisborne area has been reported by Sinclair and Simpson (1954). Trials in 1954 and 1955 have shown that, compared with perennial-ryegrass pastures, on which only slight live-weight gains were made, hoggets set-stocked at the rate of 10 per acre on clover pastures, in 4 trials, gained at the rate of approximately 0.4 lb a day over periods of 2½-3 months (Sinclair *et al.* 1956). In each of the two seasons mentioned, two consecutive trials were carried out, the first commencing in late January and the second about mid April. Each year parasite levels were much higher in the first trial but better growth rates were obtained at this time of the year. In each of the four trials worm counts by species were made on a random half of the hoggets from each treatment at the conclusion of the trials when the animals were killed at the meat works. Mean values for these worm counts have been published (loc. cit). Fig. 15 shows the mean live weights and egg counts of two groups in the first trial 1954. Tables 4A and 4B show the parasite levels, along with live-weight gains, carcass weights, and commercial grade of individual hoggets chosen at random in the first trial of 1954. No drenches were used during the trial.

TABLE 4a. PARASITE BURDENS, LIVE-WEIGHT GAIN AND CARCASS-WEIGHT OF A RANDOM NUMBER OF HOGGETS FROM THE WHITE CLOVER GROUP (Autumn 1954)

L.W. Gain in 77 days (lb.)	Carcass Weight	Grade	Abomasum			Small Intestine			
			Haemon.	*Oster.*	*T. axei*	*Trich.* spp.	*Coop.*	*Nem.*	*Bun.*
30	36	P	Neg.	400	1,100	4,700	1,000	2.300	Neg.
35	43	P	Neg.	2,100	400	5,400	900	100	Neg.
39	52	P	Neg.	400	800	5,300	600	Neg.	Neg.
34	41	P	140	600	800	2,500	Neg.	Neg.	Neg.
37	45	P	340	1,800	900	10,400	Neg.	1,400	Neg.
29	34	P	Neg.	200	100	2,800	300	Neg.	Neg.
36	46	P	80	700	300	9,600	1,600	600	2
25	39	P	Neg.	300	700	3,500	1,100	Neg.	Neg.
34	48	P	Neg.	1,500	Neg.	14,600	Neg.	Neg.	Neg.
26	35	P	20	300	400	1,600	500	100	Neg.
Mean: 32.5	43.3		58	830	550	6,040	600	450	.2

P = Prime Crossbred.
2 = 2nd Quality.

TABLE 4b. PARASITE BURDENS, LIVE-WEIGHT GAIN AND CARCASS-WEIGHT OF A RANDOM NUMBER OF HOGGETS FROM THE PERENNIAL RYEGRASS GROUP (Autumn 1954)

L.W. Gain in 77 days (lb.)	Carcass Weight	Grade	Abomasum				Small Intestine		
			Haemon.	*Oster.*	*T. axei*	*Trich.* spp.	*Coop.*	*Nem.*	*Bun.*
3	33	2	720	6,100	2,400	11,800	1,700	100	Neg.
14	30	2	Neg.	1,200	1,000	9,700	1,100	Neg.	Neg.
20	37	P	20	500	1,100	6,200	1,200	100	Neg.
16	44	P	20	1,900	2,200	11,000	1,100	Neg.	Neg.
20	31	2	Neg.	400	1,500	8,500	500	Neg.	Neg.
16	32	2	Neg.	Neg.	100	8,300	100	Neg.	Neg.
12	34	P	Neg.	100	1,700	3,700	400	Neg.	Neg.
12	29	2	80	4,700	2,000	6,800	Neg.	Neg.	Neg.
10	26	2	Neg.	100	1,000	2,100	100	Neg.	1
16	31	2	Neg.	2,100	400	2,400	300	Neg.	Neg.
13	34	2	Neg.	1,000	1,400	2,500	Neg.	Neg.	Neg.
Mean: 13.8	33.3		76	1,646	1,346	6,636	591	18	.1

P = Prime Crossbred.
2 = 2nd Quality.

The mean egg counts in Fig. 15 indicate the build up of parasitism from negligible levels to moderate levels over the period of the trial with no difference developing between the two groups. Live-weight increases in the two groups have been markedly different, however, and in this and in the other three trials show no correlation with egg counts. In the clover group (Fig. 15) except for the weight on 1 March, the live weights for the 77 days of the trial follow a typical uniform curve and show no effects from the varying parasitic levels as indicated by egg counts.

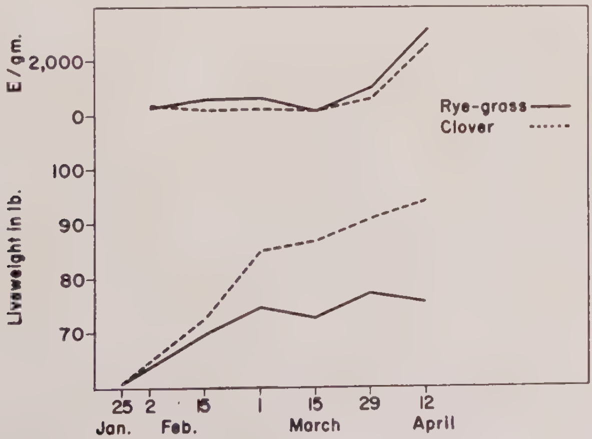


Fig. 15.—Mean live weight and mean worm egg count of hoggets on ryegrass and on white clover.

In both groups individual records throughout the trial show a purely random association between the growth curves and egg counts. Many of the hoggets building up to the highest egg counts (up to 14,000 e/g) have made the highest live-weight gains. The worm burdens carried by the lambs at slaughter likewise fail to correlate with live-weight gains, carcass weight, and grade, and individuals carrying parasite levels frequently held to be pathogenic have made excellent live-weight gains and finished as best-quality export carcasses.

Subsequent work with pen-fed animals (loc. cit.) has shown that the better growth of hoggets on white clover can be explained by an intake of digestible dry matter twice that of those on perennial ryegrass. At this high level of nutrition, parasite levels moderately high to high, and in some individuals at levels which are commonly said to be pathogenic for some of the nematode species present, have failed to impair the growth rate of the lambs.

The changing levels of parasites in the free-grazing animal are difficult to follow accurately because of such factors as the dynamic nature of worm populations, the different biotic potentials of the various species of worms and the changing environmental conditions for the free-living and parasitic stages of the parasites. Nematode egg counts obviously give only an approximate and imperfect picture. Post-mortems reveal the situation obtaining at the time of slaughter or death only. In spite of such imperfections, the data presented above and from the trials previously reported strongly suggest that the concepts of sub-clinical, clinical, and pathogenic levels of infestation, however accurately these levels are determined, must be accepted with considerable reserve. We believe that too frequently the pathogenicity of parasites has been assessed from the levels present in hoggets doing poorly from causes other than parasites and that a different concept of the pathogenicity of nematodes is derived when the burdens in thrifty sheep are examined. Our work suggests that in unthrifty hoggets parasitism is seldom the primary cause but is to be regarded as capable of further depressing the health of the unthrifty animal and at times causing death. The only exception to this that we have encountered is *H. contortus* which would seem frequently to play a primary role and to produce an ill-thrift in hoggets on what would appear, in the present state of knowledge, to be the highest planes of nutrition. We would emphasise, however, that, although in the light of our experience all nematodes other than *H. contortus* can be placed in a secondary role in the hogget ill-thrift syndrome, it is indeed possible that under certain favourable environmental conditions other species such as *Ostertagia* spp. and *Nematodirus* spp. might build rapidly to populations that could be a primary cause of ill-thrift.

References

Andrews, J. C. 1939: *J. agric. Res. 58:* 761.
Bennetts, H. W. 1933: *J. Dep. Agric. W. Aust. 10:* 240.
Bremmer, K. C. 1955: *Aust. J. Zool. 3:* 312.
Clarke, E. A.; Filmer, D. B. 1958: *N.Z. J. agric. Res. 1:* 217.
Clunies Ross, I. 1931: *Aust. J. exp. Biol. med. Sci. 8:* 217.
——— 1932: *J. Coun. sci. industr. Res. Aust. 5:* 73.

CLUNIES ROSS, I.; GRAHAM, N. P. H. 1932: *Ibid. 5:* 31.

CLUNIES ROSS, I.; CHAMBERLIN, W. E.; TURNER, HELEN NEWTON 1937: *Ibid. 10:* 313.

CLUNIES ROSS, I.; GORDON, H. McL. 1933: *Aust. Vet. J. 9:* 100.

CULBERTSON, J. T. 1941: "Immunity Against Animal Parasites". Columbia University Press, New York.

EWER, T. K. 1946: *N.Z. J. Agric. 73:* 559.

EWER, T. K.; SINCLAIR, D. P. 1951: *N.Z. J. Sci. Tech. A32 (5):* 35.

FRANKLIN, M. C.; GORDON, H. McL.; McGREGOR, C. H. 1946: *J. Coun. sci. industr. Res. Aust. 19:* 46.

FRASER, D.; ROBERTSON, A. H. H. 1933: *Emp. J. exp. Agric. 1:* 17.

GORDON, H. McL. 1948: *Aust. vet. J. 24 (2):* 17.

——————1950: *Ibid. 26 (2–5).*

GORDON, H. McL.; WHITLOCK, H. V. 1939: *J. Coun. sci. industr. Res. Aust. 12:* 50.

ROBERTS, E. J. 1933: *J. Minist. Agric. 40:* 337.

ROBERTS, F. H. S. 1942: *Aust. vet. J. 18:* 19.

ROBERTS, F. H. S.; TURNER, HELEN NEWTON; McKEVETT, MARGOT 1954: *Aust. J. Zool. 2:* 275.

SINCLAIR, D. P.; SIMPSON, J. E. V. 1954: *Proc. 16th Conf. N.Z. Grassl. Ass.* p. 101.

SINCLAIR, D. P.; CLARKE, E. A.; FILMER, D. B. 1956: *Proc. N.Z. Soc. Anim. Prod. 16:* 23.

SPEDDING, C. R. W. 1952: *Emp. J. exp. Agric. 20:* 79.

STEWART, D. F. 1955: *Nature, Lond. 176:* 1273.

TETLEY, J. H. 1941: *Cornell Vet. 31 (3).*

——————1949: *N.Z. Dep. sci. industr. Res. Bull. 96.*

WHITTEN, L. K. 1954: *Proc. N.Z. Soc. Anim. Prod. 14:* 84.

A COMPARISON OF PHOSPHATIC FERTILISERS

By N. A. CULLEN, Invermay Research Station, Mosgiel

(Received for publication, 6 December 1957)

Summary

A trial was laid down in February 1951 at Invermay Research Station to investigate pasture responses from several forms of phosphatic fertilisers in the presence and absence of lime.

The phosphatic fertilisers used were superphosphate, serpentine superphosphate, Thermophos, and North African and Nauru ground-rock phosphates. Lime treatments were: nil, 10 cwt, and 20 cwt annually.

Over a five-year period, Thermophos and serpentine superphosphate gave similar yields to superphosphate when compared on the basis of equivalent amounts of P_20_5 per acre.

North African phosphate proved comparable to superphosphate in the absence of lime, but inferior in the presence of lime. Nauru rock phosphate proved inferior to the other treatments.

Lime increased yields slightly in the superphosphate, serpentine superphosphate, and Thermophos plots, but depressed yields in the North African and Nauru rock-phosphate treatments.

INTRODUCTION

About the end of 1950 a world-wide sulphur shortage developed as a result of greatly increased consumption combined with exhaustion of some of the main sulphur deposits in America.

As New Zealand is dependent on overseas supplies of sulphur for the manufacture of sulphuric acid used in the preparation of superphosphate, a shortage of sulphur could seriously disrupt the fertiliser industry. A search for non-sulphurous alternatives to superphosphate thus became a matter of national importance, and trials were laid down over a wide range of soil types. These trials included North African and Nauru ground-rock phosphates, basic slag, serpentine and lime-reverted superphosphates, and several calcium-magnesium fused phosphates recently developed in America.

One of these, Thermophos, had previously shown promise in preliminary trials in New Zealand (Lynch 1951).

EXPERIMENTAL

Details of Fertilisers Used

Serpentine superphosphate is made by combining three parts of superphosphate with one part of serpentine rock. The resultant dicalcic phosphate is a non-acid fertiliser of excellent physical texture (Holford 1943; Grimmett and Elliott 1951). Elliott (1944) found over a wide range of soils that serpentine superphosphate was comparable to superphosphate on an equivalent-weight basis. More recent

N.Z. J. agric. Res. 1: 418–431.

results from mowing trials, however, suggest that serpentine superphosphate is comparable to superphosphate only on a basis of equivalent P_2O_5 content (Lynch 1951; Van der Elst and Karlovsky 1953). Trials in England showed serpentine superphosphate to be equal or in some cases superior to superphosphate for reseeding pasture on acid hill soils (Cook 1956). However, serpentine superphosphate generally proved inferior under Australian conditions (Grimmett and Elliott 1951).

Thermophos is one of a group of fused calcium-magnesium phosphates manufactured by melting a magnesium silicate such as olivine or serpentine with phosphate rock in an electric-arc furnace and rapidly cooling the melt in high-pressure streams of water. The process was developed by the Tennessee Valley Authority in America (Walthall and Bridger 1943) and has since been used commercially in both America and Japan (Kusugai *et al.* 1949; Huang 1953; Bridger 1951). "Thermophos" is the trade name for a fused phosphate formerly manufactured by the Permante Metals Corporation in California, U.S.A., from serpentine and Idaho rock phosphate.

Calcium magnesium phosphate is a granular greenish material with grains about the size of coarse sand (Hill *et al.* 1948). When finely pulverised it is highly soluble in 2% citric acid, though insoluble in water. Its total P_2O_5 content is about 23%.

As calcium magnesium phosphate is non-corrosive and free-running, and does not cake or absorb water, it is ideal for bulk handling and aerial distribution.

It is a basic phosphate which is most suited to acid and neutral soils, its effectiveness being reduced in alkaline areas (Huang 1953). Results from three pot trials conducted in Norway showed Thermophos to be superior to monocalcic phosphate on acid soils of pH 4.5–5.5 but not on limed soils with a pH from 6.2–6.8 (Cook 1956).

Results in New Zealand have indicated that Thermophos is equivalent or slightly superior to superphosphate when applied at equivalent P_2O_5 content on slightly acid soils. (Lynch 1951; Van der Elst and Karlovsky 1953; Karlovsky 1957). Calcium magnesium phosphate has an important liming value, one ton of the material supplying the equivalent of about three-quarters of a ton of lime.

Since these fused phosphates contain a high percentage of readily available magnesium they are likely to be of particular benefit on magnesium-deficient areas.

North African Rock Phosphate (*Gafsa*) has been used in high-rainfall districts in New Zealand for many years, mainly on acid hill soils where it has compared favourably with superphosphate. Results have been poor, however, on limed soils and in drier districts (Lynch 1951).

In English trials Cook (1956) found ground North African rock phosphate to be less effective than superphosphate for establishing pasture and on established grassland even under acid conditions.

Nauru Rock Phosphate is a high-grade rock containing about 36% total P_2O_5 of which up to 8% is citric soluble. Ground Nauru rock phosphate has been included in a number of trials in New Zealand, but results generally have been poor, Nauru being inferior to superphosphate and even North African rock phosphate in most trials (Lynch 1951; Cottier 1952; Cullen 1954). Mixtures of Nauru rock with superphosphate have also been tried but have not given encouraging results (Cottier 1952).

Design of Trial

A replicated mowing trial was laid down at Invermay Research Station in March 1951 incorporating the following treatments:

1. Control—no phosphate
2. Superphosphate 3 cwt per acre per annum
3. North African phosphate (Gafsa) 3 cwt per annum
4. Ground Nauru rock phosphate 3 cwt per annum
5. Ground Nauru rock phosphate 10 cwt initially and 3 cwt per annum subsequently
6. Thermophos } equivalent P_2O_5 content to 3 cwt
7. Serpentine superphosphate } superphosphate per annum

Lime (pure carbonate basis), (a) Nil, (B) 10 cwt per annum, (C) 20 cwt per annum.

A basal dressing of 1 cwt muriate of potash was applied over the whole trial at laying down.

A split-plot latin-square design was chosen with the lime treatments as the main plots and the phosphatic fertilisers as the sub-plots. Plot size was 30 x 6 ft. This allowed for two 2-ft cuts to be taken from each plot. The technique adopted was "mowing and clippings returned" (Lynch 1947). This technique is suitable for fertiliser trials as no transference of fertility can occur between plots while a good measure of the relative production is obtained.

The trial was laid down on a three-year-old sward in which ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) were the dominant species and cocksfoot (*Dactylis glomerata*), Montgomery red clover (*Trifolium pratense*), and weed grasses subdominants. The clovers were rather pale and unthrifty. This was subsequently shown to be due to molybdenum deficiency.

After spectacular molybdenum responses were obtained at the Station in 1951 (Holmes *et al.* 1951), a basal dressing of $1\frac{1}{4}$ oz/acre of sodium molybdate was applied to the trial in September 1951.

Soil Tests

Soil samples were drawn from all treatments in 1954, 1955, and 1956 and tested for pH levels and for available phosphate by the method of Truog (1930).

Soils

The trial area is located on a level site in the lower foothills at Invermay Research Station at an altitude of approximately 300 ft.

The soil is a silt loam overlying clay similar to the Warepa series of yellow-grey earths which occur throughout South Otago. The parent material is mainly phonolitic volcanic rock which has been covered in most places by loess soils deposited to varying depths.

The texture of the soil is inclined to be heavy, leading to impeded drainage and cultivation difficulties.

In its natural state the nutrient status is low, the soil being deficient in calcium, phosphorus, and molybdenum. No marked sulphur deficiency has been demonstrated.

The trial site had received some lime and phosphatic fertiliser prior to 1951, but despite these dressings the calcium and phosphorus levels were low and the area was considered suitable for investigating phosphate and lime responses and interactions.

Climate

The annual rainfall average for the district is approximately 27 in. Although distribution is fairly even throughout the year, droughts are experienced periodically during the summer months. Growth normally commences during September, reaches its peak about the end of November, and falls off during January and February. A slight autumn flush may occur during March and April, but growth has usually ceased by May except for short-rotation ryegrass and other winter-growing species.

The normal pattern of growth results in grasses dominating during the spring and clovers in the summer. Phosphatic fertiliser applied during the late summer and autumn months has been shown to result in improved winter and early spring growth (Cullen 1954), and the fertiliser applications were made in March for this reason.

RESULTS

Five seasons' results are now available from this trial. Excellent phosphate responses were obtained throughout the five years, superphosphate, Thermophos and serpentine superphosphate, proving superior to the rock phosphates. North African phosphate was almost as good as the more soluble phosphates in the absence of lime, but yields were inferior in the presence of lime. Nauru rock phosphate even at the 10 cwt initial application rate gave poor results generally, inferior to North African rock phosphate.

Lime at both rates increased yields slightly in the superphosphate, Thermophos and serpentine superphosphate plots, but depressed yields considerably in the rock-phosphate treatments.

During the first two years, samples taken for dry matter determination were bulked from the three replicas of each treatment and statistical analysis of the dry-matter yields was therefore not possible. Although the green weights have been subject to statistical analysis, these results are somewhat misleading because of differences in dry-matter percentages between treatments. The dry-matter percentage

TABLE 1. SUMMARY OF DRY MATTER YIELDS (lb/ac) RELATIVE TO NO PHOSPHATE NO LIME (100)

Treatment	51/52	52/53	53/54	54/55	55/56	Total D.M.	
						(lb/ac.)	(rel.)
No Lime							
No phos.	100	100	100	100	100	24,270	100
Super.	133	160	202	157	135	38,240	158
N. Afr.	134	144	192	161	133	37,060	153
Nauru 3	118	125	158	146	135	33,160	137
Nauru 10	130	141	174	149	131	35,240	145
Thermophos	138	160	204	168	138	39,280	162
Serp. super	127	147	200	164	142	37,960	156
10 cwt Lime							
No phos.	103	101	130	123	117	27,820	115
Super.	133	175	219	178	148	41,630	172
N. Afr.	110	123	166	148	135	33,210	137
Nauru 3	114	121	151	137	123	31,370	129
Nauru 10	117	132	168	146	118	33,110	136
Thermophos	133	162	222	177	141	40,630	167
Serp. super.	129	153	214	170	133	39,270	162
20 cwt Lime							
No phos.	96	99	133	126	116	27,710	114
Super.	138	168	219	167	134	40,120	165
N. Afr.	111	119	174	146	136	33,220	137
Nauru 3	104	106	146	128	128	29,690	122
Nauru 10	114	128	178	151	132	34,200	141
Thermophos	135	163	224	175	131	40,240	166
Serp. super.	128	159	221	176	147	40,410	167
Sig. dif. 5%	Not Available		16	12	13	Not Available	
1%			21	16	18		

of the control plots was normally much higher than that from the more efficient phosphate treatments.

Significant differences are quoted only for the years in which statistical analysis of dry weights was possible. The figures quoted are for comparisons between phosphate treatments with the same lime treatment.

Response to the Various Fertilisers

Thermophos proved as good as superphosphate in this trial both in the acid no-lime treatments and in the limed plots. At no time were marked differences apparent either in seasonal or yearly production between these two fertilisers.

Serpentine superphosphate also proved comparable to superphosphate over the five year period, being slightly inferior in the first two years but at least as good in the final years. However, it must be remembered that Thermophos and serpentine superphosphate were compared with superphosphate on an equivalent P_2O_5 basis, not on an equal-weight basis.

North African phosphate proved comparable to superphosphate only in the absence of lime, yields being depressed considerably by both rates of lime.

Nauru rock phosphate was inferior to North African rock phosphate at similar rates, and even at 10 cwt initially and 3 cwt subsequently proved no better than 3 cwt of North African rock phosphate. At some cuts the Nauru plots yielded little more than the controls.

On this soil type it is evident that Nauru rock phosphate even when applied at high initial rates is of limited value and offers little promise as a substitute for superphosphate.

Results for Each of the Five Seasons

1951–52. Phosphate yields were about 30% above control, Thermophos and superphosphate being slightly but significantly better than serpentine superphosphate (in green yields).

The rock-phosphate treatments compared favourably with Thermophos and superphosphate in the no-lime plots, but were significantly poorer in the presence of lime.

1952-53. Excellent responses were noted, especially in the superphosphate, Thermophos and serpentine superphosphate plots. The rock-phosphate treatments continued to yield well (though less than superphosphate) in the unlimed plots but poorly in both limed series. Serpentine superphosphate was again slightly inferior to superphosphate and Thermophos.

1953-54. The best treatments yielded more than double control, differences being greatest in the late autumn, winter, and early spring. In this season the limed control plots for the first time showed a significant increase in dry-matter yields compared with the unlimed controls. Dry-matter yields from the superphosphate, Thermophos, and serpentine superphosphate plots were comparable; a significant depression again occurred in the North African plots.

1954-55. Although the magnitude of response was less than the previous year, differences were still marked. The prevoius year's trends continued, with superphosphate, Thermophos, and serpentine superphosphate showing a response to lime, and North African and Nauru rock phosphates the usual decrease in the presence of lime. The limed controls again yielded significantly higher than those without lime.

1955-56. Dry conditions throughout the summer and autumn led to reduced yields and smaller phosphate responses. In the no-lime series, differences between the phosphate treatments were small. In the 10 cwt lime series, superphosphate gave the best response, being significantly better than serpentine superphosphate. However, in the 20 cwt lime series both superphosphate and Thermophos were significantly (5%) poorer than serpentine superphosphate, while superphosphate was significantly poorer in the 20 cwt than in the 10 cwt lime series. The reason for this is not clear.

TABLE 2. SEASONAL DRY-MATTER PRODUCTION. RELATIVE TO SUPERPHOSPHATE (100)

Treatment	Spring	Summer	Autumn	Total D.M.	
				(lb/ac.)	(rel.)
No Lime					
No phos.	65	68	47	24,270	63
Super.	100	100	100	38,240	100
N. Afr.	96	100	91	37,060	97
Nauru 3	88	90	71	33,160	87
Nauru 10	92	96	81	35,240	92
Thermophos	102	104	101	39,280	103
Serp. super.	102	96	99	37,960	99
10 cwt Lime					
No phos.	71	69	45	27,820	67
Super.	100	100	100	41,630	100
N. Afr.	83	83	59	33,210	80
Nauru 3	78	80	53	31,370	75
Nauru 10	82	83	63	33,110	80
Thermophos	96	102	95	40,630	98
Serp. super.	93	94	92	39,270	94
20 cwt Lime					
No phos.	73	74	45	27,710	69
Super.	100	100	100	40,120	100
N. Afr.	86	86	65	33,220	83
Nauru 3	79	78	49	29,690	74
Nauru 10	88	89	66	34,200	84
Thermophos	97	108	95	40,240	100
Serp. super.	100	103	98	40,410	101

Seasonal Responses

This table indicates that the response to serpentine superphosphate and Thermophos during the spring, summer and autumn was as good as or better than that from superphosphate.

In most years responses have followed a fairly consistent pattern. Greatest phosphate responses have been noted during the late autumn, winter, and early spring, when growth is at a minimum. At this time, production from the best treatments may be several times that of the control, e.g., a cut taken during June 1953 showed production from the superphosphate, Thermophos, and serpentine superphosphate treatments to be more than three times that from the control treatment. At the time of cutting it was observed that short-rotation ryegrass was contributing the bulk of the herbage in these plots.

During the spring months, when growth was at a maximum, responses to all phosphatic treatments were small. During October in 1954 and 1955 the phosphate response was less than 10%. At this time of the year grasses assume dominance and even the normally low-producing weed grasses provide a large bulk of herbage. The folly of relying entirely on results from one or two hay cuts taken at a time when differences are normally at a minimum, is apparent. During the summer, a response intermediate between that of the out-of-season cuts and the peak spring-growth period was normally recorded, the extent of the response being largely dependent on weather conditions. Under abnormal drought conditions, little if any response may be recorded. This would be expected where all herbage is dried off.

Lime Responses

No marked response to either rate of lime was noted in the first season, though a yield depression was apparent in the rock-phosphate treatments. In the second season there was a slight lime response in the superphosphate and serpentine superphosphate treatments, while the depression in yield continued in the rock-phosphate treatments.

In the third and fourth years, superphosphate and serpentine superphosphate responded considerably to lime, and Thermophos to a lesser degree. The control plots showed a marked lime response in the third, fourth, and fifth years.

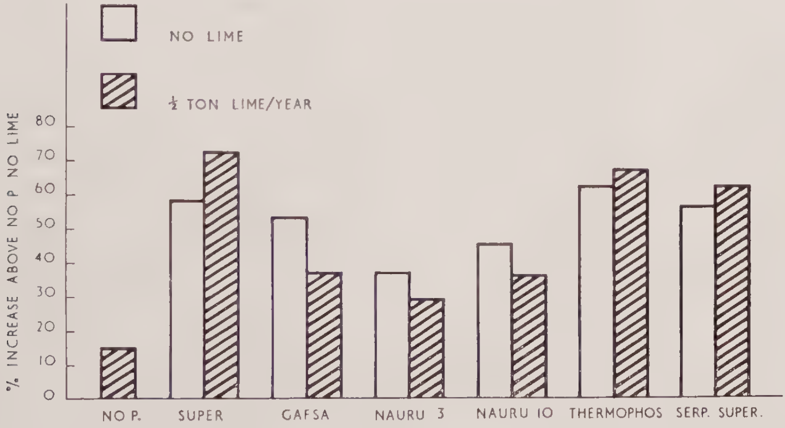


Fig. 1 —Effect of 10 cwt calcium carbonate on phosphate yields (1951–52 to 1955–56)

No differences have been noted between the two rates of lime. This is perhaps not surprising as the low rate was comparatively high (10 cwt/year), but in the later years at least it might have been expected that the high rate would have affected the rock phosphates more than the low rate. This did not prove to be the case.

Over the five-year period lime gave a 12% increase in the superphosphate plots, a 9% increase in the serpentine superphosphate plots, and a 5% increase in the Thermophos plots.

However, with North African phosphate, lime caused a 16% reduction; with Nauru 3 cwt, an 11% reduction; and with Nauru, 10 cwt initially and 3 cwt subsequently, a 6% reduction. Since North African rock phosphate without lime was the best of the rock phosphates, it had most to lose by liming. It can be seen from the above that this is exactly what happened.

Botanical Composition and Sward Changes

Despite continual mowing for five seasons, there was little deterioration of sward in the efficient phosphate treatments. The only evidence of deterioration was an increase in the percentage of Yorkshire fog (*Holcus lanatus*), but this would be expected as this species is favoured by mowing and spelling.

In the limed superphosphate, serpentine superphosphate, and Thermophos plots, white clover was the dominant legume while the control and poorer rock-phosphate treatments favoured red clover.

Similarly the percentage of ryegrass was higher in the more efficient phosphate treatments, while the percentage of weed grasses was higher on the controls. Percentage differences in composition in the seven treatments can be seen from herbage dissection analyses.

Table 3 shows that, although the percentage of total grasses and of total clover is not affected by treatment, there are large variations

TABLE 3. SUMMARY OF PASTURE DISSECTION RESULTS
(Average of 15 pasture dissections)

Treatment	Ryegrass Species %	Other Grasses %	Total Grasses %	Ratio Rye/ Other Grasses	White Clover %	Red Clover %	Total Clover %	Ratio White to Red Clover
No Lime								
No lime, no phosphate	35	28	63	1.3	17	15	32	1.1
Superphosphate	42	19	61	2.1	29	8	37	3.9
Nth. African rock phosphate	40	20	60	2.0	27	12	39	2.4
Nauru rock, 3 cwt	35	26	61	1.4	27	10	37	2.8
Nauru rock, 10 cwt	37	21	58	1.8	28	11	39	2.6
Thermophos	44	17	61	2.6	28	9	37	3.1
Serp. super.	40	24	64	1.7	26	8	34	3.0
10 cwt Lime								
No phosphate	35	22	57	1.6	17	20	37	0.9
Superphosphate	46	17	63	2.7	30	5	35	5.5
Nth. African rock phosphate	40	19	59	2.1	24	14	38	1.7
Nauru rock, 3 cwt	40	20	60	2.0	24	14	38	1.8
Nauru rock, 10 cwt	39	19	58	2.0	23	16	39	1.4
Thermophos	43	16	59	2.8	32	7	39	4.5
Serp. super.	42	16	58	2.6	29	9	38	3.1
20 cwt Lime								
No phosphate	40	20	60	2.1	17	18	35	1.0
Superphosphate	44	18	62	2.5	29	8	37	3.4
Nth African rock phosphate	42	19	61	2.2	23	14	37	1.6
Nauru, 3 cwt	40	18	58	2.2	20	18	38	1.1
Nauru, 10 cwt	42	19	61	2.1	20	16	36	1.2
Thermophos	46	14	60	3.2	30	9	39	3.2
Serp. super.	45	17	62	2.7	27	9	36	3.0

in the ratio of ryegrass to other grasses and of white to red clover. These ratios increase as the yield or quality of pasture increases. Liming, however, has generally increased the ratio of ryegrass to other grasses, while the ratio of white to red clover is more sensitive to the available phosphate. On the No Lime treatments all phosphates have markedly increased the ratio of white to red clover. On the limed plots the rock phosphates have a much lower ratio of white to red clover than the other phosphates.

These herbage dissection figures indicate the major composition changes. However, herbage dissection is primarily a measure of production rather than of sward composition and hence those species in the sward which are highest producing are favoured. For example, a few ryegrass plants in a control plot may produce most of the herbage since the low-producing weeds and grasses may be largely below mower height and as a consequence contribute only a small fraction of the herbage.

For this reason botanical changes have probably been greater than is evident from dissection data. However, dissection analyses indicate that the control plots contained more flatweeds, red clover, and weed grasses, such as browntop (*Agrostis tenuis*), sweet vernal (*Anthoxanthum*

TABLE 4. INFLUENCE OF LIMING ON TRUOG TEST FOR PHOSPHATE

Treatments	Date of Sampling					
	15/2/54		28/2/55		21/2/56	
	pH	P	pH	P	pH	P
No Lime						
No phos.	5.7	4	5.6	4	5.5	3
Super.	5.5	7	5.5	6	5.5	6
N. Afr.	5.6	10	5.6	9	5.6	14
Nauru 3	5.5	18	5.6	19	5.4	36
Nauru 10	5.7	27	5.7	28	5.5	45
Thermophos	5.7	8	5.7	6	5.8	9
Serp. super.	5.5	6	5.5	5	5.5	7
10 cwt Lime						
No phos.	6.5	5	6.4	5	6.5	5
Super.	6.4	8	6.2	7	6.5	9
N. Afr.	6.5	14	6.4	19	6.6	30
Nauru 3	6.3	25	6.4	36	6.4	40
Nauru 10	6.6	34	6.6	44	6.5	52
Thermophos	6.7	7	6.6	6	6.7	8
Serp. Super.	6.5	6	6.4	8	6.4	8
20 cwt Lime						
No phos.	6.8	4	6.9	4	6.8	5
Super.	6.8	9	6.9	8	7.1	10
N. Afr.	6.9	17	7.0	20	7.1	40
Nauru 3	6.8	26	7.0	28	7.1	45
Nauru 10	6.9	38	6.9	45	6.8	58
Thermophos	7.1	9	6.9	10	6.9	10
Serp. super.	6.8	8	6.8	9	7.0	10

NOTE.—P = parts of available phosphate per 50,000,000 of soil extract.

odoratum), and Yorkshire fog (*Holcus lanatus*), than the higher-yielding phosphate treatments. These latter treatments also contained a high proportion of short-rotation ryegrass (*Lolium multiflorum* × *L. perenne*) which appeared to have increased as a result of the build up in fertility. The presence of short-rotation ryegrass may explain the relatively better yields of the efficient phosphate treatments in the winter and early spring.

Summary of Soil Analysis Results

Comments on Table 4

In spite of the annual applications of 10 cwt and 20 cwt lime per acre, the pH did not rise during the three years for which soil analyses are available.

The following are the average phosphate figures over the three years:

	Lime	*P Value (Truog)*
Average of superphosphate serpentine superphosphate, and Thermophos	Nil	6.7
	10 cwt	7.6
	20 cwt	9.2
Average of Nauru and North African rock phosphates	Nil	23
	10 cwt	33
	20 cwt	35

The rise in the phosphate figure due to liming is more marked in the rock-phosphate treatments. The change occurs largely between the no-lime and 10 cwt rates. This change corresponds to a lowering of efficiency of rock phosphates.

The phosphate figures have risen steadily in the rock-phosphate plots but not in the plots receiving the more soluble phosphates.

Discussion

In evaluating various forms of phosphates, care is necessary to see that results are not influenced by the presence of major or minor elements in the fertiliser.

Many claims for superiority of slag can be explained by its trace-element content. Recent research in New Zealand has indicated that the molybdenum, vanadium, and tungsten content may separately or in combination give marked responses (Davies and Stockdill 1956; During 1955). North African phosphate has a relatively high molybdenum content (4.0 p.p.m.), and this could be responsible for good responses with this fertiliser on molybdenum-deficient country.

Sulphur deficiency in New Zealand and interactions have been described by Walker *et al.* (1955) who suggest a new phosphate evaluation in the light of recent knowledge. In the experiment described however, Thermophos has compared favourably with superphosphate over a five-year period and it is unlikely that sulphur has influenced the trial results to date. Although one advantage claimed for serpentine superphosphate is that it has a high content of magnesium, this element does not appear to have been of benefit in this trial.

Both magnesium and sulphur are included in a neighbouring trial on the same soil type and have shown negligible responses in three years' yield measurements.

The rates of lime chosen may appear unduly high. This trial was planned at a time when the use of heavy lime applications was general in the district, little being known then of molybdenum deficiencies. It has since been shown that much of the benefit of heavy lime dressings is to make molybdenum available (Davies 1956).

With 20 cwt lime per acre, a pH of about 6.9–7.0 was reached in the third year after application. The pH tended to remain at this level despite further annual lime applications.

With 10 cwt lime the pH level also remained relatively stationary at about 6.4.

The increased lime response in the control treatments has already been mentioned. The extent of this response is surprising, being about 30% in the third season and over 20% in the fourth.

The reason for this increase is not clear. The most likely explanation is an increase in molybdenum availability together with a release of phosphate (there has been some evidence of the latter in the soil-test figures). A basal dressing of $1\frac{1}{4}$ oz sodium molybdate was applied in September 1951 and the increases were noted in the 1953-54 season—over two years later. Other work at Invermay Research Station (Cullen 1955) has indicated that responses from 1 oz sodium molybdate continue for approximately three years, and it is quite probable that this response from both rates of lime was largely an indirect molybdenum effect. The measurement of responses generally proved satisfactory and it is considered the technique adopted was suitable. Normally the plots were cut six to seven times each season when the herbage was three to five inches in height and capable of being cut by a Dennis motor mower.

At certain times hay cuts were necessary because of wet weather interfering with normal cutting but these were avoided whenever possible because of the possibility of sward upsets.

When the sward is left for a lengthy period in a long and rank state the control plots tend to continue growing relatively faster than the higher-producing treated plots. The latter may lodge and almost cease growth. If left unduly long prior to cutting the controls may even outyield the treated plots.

In such instances recovery following cutting may be much slower on the treated plots. This is especially so where the herbage in the control plots is short and dense and in the topdressed plots rank and open at the base. As the herbage is normally removed from the plots when they are hayed, the sward is left open and is liable to dry out. This occurred in the 1955 season when dry conditions resulted in very poor recovery following haying. As a result the control plots outyielded the treated ones at two subsequent cuts and the poorer treatments appeared in a very favourable light. It can be seen that false interpretation could be given to yield data obtained under such conditions.

Results from this trial have in the main confirmed work carried out in New Zealand and elsewhere. Superphosphate proved superior to the other phosphates in this trial, under acid and neutral soil conditions. Thermophos and serpentine superphospate proved equal to superphosphate on an equivalent P_2O_5 basis only. North African phosphate applied at equivalent weight was almost as good as superphosphate under acid soil conditions ($pH<6$) and could be used as an alternative on acid soils. Results from Nauru rock phosphate were generally poor, and it is doubtful if this phosphate is worthy of serious consideration as a substitute. The superiority of North African phosphate over Nauru is probably due to the former being a much softer rock. Both rock phosphates have a relatively low fluorine content. Although Thermophos compared favourably with superphosphate in the trial, it has the disadvantage of containing no sulphur. As sulphur deficiencies appear to be widespread in New Zealand, serious consideration would be necessary before recommending the use of phosphates without sulphur. The fused phosphates could possibly replace superphosphate on soils where sulphur was known to be in adequate supply.

ACKNOWLEDGEMENTS

Thanks are due to Mr. R. S. Scott who has been responsible for this trial during the past two years; to Messrs. R. Abernethy, J. Flewitt, and others who helped with the field work; to Miss V. McLean and her assistants for the herbage dissection analyses; and to the statistical section of the Extension Division who were responsible for the compilation of much of the data shown in the tables.

REFERENCES

Bridger, G. L. 1951: "Fertilizer Production in New Zealand." Government Printer, Wellington, N.Z.

Cook, G. W. 1956: Field Experiments on Phosphate Fertilisers. *J. agric. Sci. 48 (1):* 74-103.

Cottier, K. 1952: Trials with Nauru Rock Phosphate as Pasture Topdressing. *N.Z. J. Agric. 84:* 183-9.

Cullen, N. A. 1954: Phosphate Trials at Invermay Research Station. *N.Z. J. Agric. 89:* 505.

——1955: Trace Element Trials at Invermay Research Station. *Proc. 17th Conf. N.Z. Grassl. Ass. 1955.* p. 163.

Davies, E. B. 1956: Molybdenum Availability in Soils. *Soil Sci. 81 (3):* 209-21.

Davies, E. B.; Stockdill, S. M. J. 1956: A Pasture Response to Sodium Tungstate on a New Zealand Soil. *Nature 178:* 866.

During, C. 1955: Trace Elements for Pastures and Lucerne. *Proc. 17th Conf. N.Z. Grassl. Ass.* p. 151.

Elliott, A. G. 1944: Serpentine Superphosphate. *N.Z. J. Agric. 69:* 27-9.

Grimmett, R. E. R.; Elliott, I. L. 1951: The Economics of World Availability and Use of Fertiliser Materials. *Proc. U.N. sci. Conf. Conserv. Util. Resources 2:* 285-8.

HILL, W. L.; WARD, F. N.; ARMIGER, W. H.; JACOB, K. D. 1948: Composition and Fertiliser Value of Phosphate Rock Magnesium Silicate Glasses. *J. Ass. Off. agric. Chem. 31:* 381-97.

HOLFORD, G. H. 1943: Characteristics of Serpentine Super. *N.Z. J. Agric. 66:* 11-13.

HOLMES, G. A.; LYNCH, P. B.; DAVIES, E. B. 1951: Pasture Responses to Molybdenum Topdressing in Otago and Southland, *N.Z. J. Agric. 83:* 247-50.

HUANG, T. H. 1953: Serpentine Fused Phosphate. *Farm Chemicals 116:* 41.

KARLOVSKY, J. 1957: Fused Phosphate Fertiilsers. *N.Z. J. Sci. Tech. A38:* 770-6.

KUSUGAI, S.; NAKAMARU & NAKAGAWA 1949: Studies on Manufacture and Mineral Effects of Fused Magnesium Phosphates: *J. Soils & Fert. (Jap.) 19 (5-6):* 178-88.

LYNCH, P. B. 1947: Methods of Measuring the Production from Grasslands. *N.Z. J. Sci. Tech. A28:* 385-405.

——— 1951: Alternative Fertilisers to Superphosphate. *N.Z. J. Agric. 83:* 420-8.

TRUOG, E. 1930: *J. Amer. Soc. Agron. 22:* 847-82.

VAN DER ELST F.; KARLOVSKY J. 1953: Phosphate Fertilizers. *N.Z. J. Agric. 86:* 146.

WALKER, T. W.; ADAMS, A. F. R.; ORCHISTON, H. O. 1955: The Effects and Interaction of Sulphur, Phosphorus and Molybnedum on the Growth and Composition of Clovers. *N.Z. J. Sci. Tech. A36:* 470-82.

WALTHALL, J. H.; BRIDGER, G. L. 1943: Fertiliser by Fusion of Rock Phosphate with Olivine. *Indust. Engng. Chem. 35:* 774-7.

CORRIGENDA

Volume 1, page 152, 4th para., *Experiment 1.*

The 3rd and 4th lines of this paragraph should read:

The only significant or nearly significant responses were to P or PN, and plant analytical levels for other nutrients showed no abnormalities.

Volume 1, pages 301, 305, 329, 341:

In the bibliographical reference at the foot of each of these pages, for *N.Z. J. agric. Res. 3:* read *N.Z. J. agric. Res. 1:*

NEW ZEALAND JOURNAL OF SCIENCE

Vol. 1, No. 2, May 1958

CONTENTS

(Provisional)

Doppler Measurements on the Soviet Satellites. *A. H. Allen* and *J. E. Drummond*

Sulphur Isotopic Variations in Nature: Part 6—Sulphur Isotopic Measurements on the Discharge from Fumaroles on White Island. *T. A. Rafter, S. H. Wilson* and *W. B. Shilton*

An Attempt to Extend the Operating Temperature of Copper Cathode Geiger Tubes. *R. E. Belin* and *A. E. Bainbridge*

Coccids Associated with Rata and Kamahi in New Zealand. *J. M. Hoy*

Immature Stages of *Eriococcus orariensis* Hoy (Homoptera Coccidae). *J. M. Hoy*

Marine Fouling at the Port of Lyttelton. *T. M. Skerman*

The Spectrophotometric Determination of Molybdenum, Tungsten, and Vanadium in Soils and Plants. Part 1—In Soils. *J. H. Watkinson*

Some Relations to be Expected Between the Directional Spectra of Swell Observed at Different Times and Places on the Ocean. *N. F. Barber*

An Investigation of Some New Zealand Sands for Foundry Moulding. *J. E. Scahill*

Podocarpus spicatus Heartwood Extractives. *H. V. Brewerton*

Incidence of Coccidia (Sporozoa) in Wild Rabbits *Oryctolagus cuniculus* (L) in Hawke's Bay, New Zealand. *P. C. Bull*

The Heat Pulse Method for Measuring the Rate at which Sap is Flowing through the Stem of a Plant. *R. L. Closs*

CONTENTS

PAGE

Some Effects of MCPB on Establishing Pastures. *D. B. Edmond* 281

Laboratory Methods for Measuring the Toxicity of Pesticides to Honey Bees. *T. Palmer-Jones* 290

The Relationship of *Olpidium brassicae* (Wor.) Dang. to the Big-vein Disease of Lettuce. *P. R. Fry* 301

The Tiller Population of Pastures. *K. J. Mitchell* and *A. C. Glenday* 305

The Influence of Treading on Pasture: A Preliminary Study. *D. B. Edmond* 319

Effects of Flowering on the Root System and Summer Survival of Ryegrass. *Kathleen Soper* 329

Seasonal Growth Relationships in Aberdeen Angus × Jersey Crossbred Cattle. *M. A. MacDonald* 341

Bloat in Cattle. VIII. Prevention by Pasture Spraying with Anti-foaming Agents. *C. S. W. Reid* 349

The Effect of Thyroxine on Wool Growth and Lamb Production. *I. E. Coop* and *V. R. Clark* 365

Studies in Hogget Rearing. II. The Role of Parasites in Hogget Ill-thrift. *E. A. Clarke* and *Daisy B. Filmer* 382

A Comparison of Phosphatic Fertilisers. *N. A. Cullen* 418

SUBSCRIPTIONS. **Subscriptions will be accepted only on advance payment and only for a full calendar year. Price: 30s. per annum; single copy 5s.; post free (surface mail). Published in alternate months, starting February. Subscriptions should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 8018, Wellington, New Zealand. Cheques should be made payable to the Department of Scientific and Industrial Research, and if drawn on banks outside Wellington, must have exchange added. Subscribers are requested to give their name and full postal address.**

This journal may also be ordered through The High Commissioner for New Zealand, 415 Strand, London W.C.2, or through any bookseller.

Printed by Coulls Somerville Wilkie Ltd.,
Wellington, New Zealand.

New Zealand Journal of Agricultural Research

~~NCF~~

VOLUME I No. 4

AUGUST 1958

COMMONWEALTH MYCOLOGIC
26 SEP 1958

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind, however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. 4¼ × 7 in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is 4¼ × 7 in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation, etc., of references, see examples in this issue.

NEW ZEALAND JOURNAL
of
AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. A. Black.

VOL. 1. AUGUST 1958 Number 4

PHOTOSENSITIVITY DISEASES IN NEW ZEALAND
XII. CONCENTRATION OF THE FACIAL ECZEMA POISON

By E. P. WHITE, Ruakura Animal Research Station, Department of Agriculture, Hamilton

(*Received for publication, 23 May 1958*)

Summary

This paper describes procedures for the preparation of concentrates of the facial eczema hepatotoxin from toxic dried grass. Fractions have been made repeatedly containing 1/3000 of the weight of the original dried grass with very little loss of poison, and fractions containing 1/20,000 and up to 1/100,000 of the weight with a slight loss of the poison.

Other information from fractionation studies is given, providing alternative techniques, and giving information on the chemical and physical properties of the poison.

Evidence is presented that the concentration of the poison in relatively toxic dried grass is well below the order of 1 part in 100,000.

INTRODUCTION

The presence in New Zealand pastures growing under autumn flush conditions of a poison which causes the disease facial eczema in sheep and cattle has been inferred from the nature and occurrence of the disease (Filmer 1951; Clare 1952, 1955). Liver damage characteristic of facial eczema has in fact been produced in lambs and guinea pigs fed dried grass obtained from pasture that produced liver damage in lambs grazed upon it (Simpson *et al.* 1957).

Earlier attempts to isolate a poisonous principle have been delayed because of the difficulty of collecting and preserving toxic grass, and by the lack of a suitable small scale test animal for measuring toxicity. Clare (pers. comm.) in 1949 produced in a lamb slight liver damage, which could not be attributed specifically to the facial eczema poison, by dosing the alcoholic extract of 150 lb of toxic grass over 15 days.

In 1952, following the demonstration that the guinea pig could be used to test for the poison by Evans *et al.* (1957), work on isolation of the active principle was commenced using grass collected the year before.

N.Z. J. agric. Res. 1: 433–446.

The work of Perrin (1957) on the susceptibility of the guinea pig to the poison, done concurrently with the extraction work, has allowed feeding techniques for extracts to be improved during the course of the work. This paper reports the progress so far in the systematic extraction and fractionation of the poison from pasture, and decribes the procedures that have been employed.

During 1952 it was established that ether or acetone extracted the poison from dried grass (Perrin *et al.* 1953) thus proving that an organic poison was involved. A few advances such as the demonstration of the need for "cold evaporation" of solvents were made prior to March 1955 when grass of higher toxicity was obtained. Most of the progress reported has been made since that date.

Throughout this work, certain general principles have been observed. Firstly, because of the small amount of toxic grass available each year, and the uncertainty of further supplies, usually only a few fractionation experiments have been done at a time. Subsequent possible steps were often developed in the meantime using non-toxic grass, and decided upon only when results of previous trials were available. Since the location of the poison in fractions required feeding a guinea pig for at least four weeks, and up to six weeks during the early stages of the work, this system has delayed progress. Recently, with more toxic grass available, many fractionations have been done at a time.

Secondly, as it was thought that the facial eczema poison was likely to be easily destroyed, and as this was evident in the early stages of the work, the simplest and least rigorous techniques were used. For example, extracts were at first dispersed directly on grass for feeding, and only later were methods of obtaining dried toxic extracts developed.

Thirdly, since until recently, grass could not be spared to obtain information on the stability of concentrates in solution or in the dried state, each fractionation experiment has started from the grass. All processes were carried out without delay, with a minimum of storage of solutions or dried extracts.

Techniques have been developed on the basis of splitting the weight of a fraction into smaller well-defined fractions, and only those in which the poison was repeatedly recoverable without loss in a small fraction, with no more than traces in the other fractions, were adopted.

EXPERIMENTAL

Grass Samples Used, and Feeding of Extracts

The nine grass samples used in this work were collected in different years from several localities, and were from ryegrass-dominant pastures (including in one case short-rotation ryegrass) and have contained small amounts of white clover and other species.

At first the grass was stored in sacks in a dry place, but the poison disappeared after eighteen months. Subsequently all toxic samples have been coarsely ground in a Wiley mill, and packed into large tins which were stored at 0 to −10°c. Under such conditions no loss of toxicity has been observed within eighteen months.

The amount of grass used in each extraction has been between one and a half and two times the quantity required to give severe liver damage when grass was fed direct. Because the samples differed considerably in toxicity, and guinea pigs of progressively lower weight were used during the course of the work, the duration of feeding and weight of grass used has varied considerably. When only low toxicity samples were available (1953, 1954) extracts of 2700 g were fed to 160-g guinea pigs for six weeks with a few positive results. For the more toxic samples (Claudelands Showgrounds 1955, 1956; Gisborne 1956, 1957) extracts of 550 or 900 g were fed to guinea pigs of 80–100 g initial weight for three weeks, followed by one week on non-toxic grass. Such extracts consistently gave severe damage.

Extracts were fed on ground grass in the manner described by Perrin (1957). Extracts in all solvents used except aqueous methanols, can be put on ground non-toxic grass in a large dish, and the solvent removed in an air-blast, with ice-cooling in some cases, without losing toxicity. Dried concentrates were generally dissolved in ether for dispersal. The weight of grass used was adjusted for the scale of feeding used, and in all later work was 168 g. This gave 21 lots of eight grammes of preparation, each of which was put in a carton and mixed with four grammes of milk powder. Usually all the preparation was consumed, and an adequate weight gain resulted. Occasionally guinea pigs did not survive the four weeks, but no deleterious effects or liver damage due to the solvents used were observed.

Originally preparations for feeding were made in several batches, but experience showed that preparations were stable at least for some weeks, so the full requirements for each guinea pig were prepared from one batch of grass, and as a precaution cartons not needed for a particular week's feeding were kept at −10°c.

Liver damage was assessed as described by Perrin (1957). In this work, visual examination and weight of livers have been the main guides, and these have been adequate. Sections have been kept for histological examination and in a few cases minor damage not detected visually has been shown histologically. Liver damage due to *Bacillus bronchoseptica* infection is easily confused visually with the predominantly necrotic type of facial eczema damage sometimes encountered. Visual examination of lungs, and histology, are important in this case.

Preparation of a × 70,000 Concentrate of the Poison

Details of the concentration procedure are given for 900 g of grass, an amount of highly toxic grass suitable for the feeding of concentrates to a single guinea pig, on the feeding scale now used. For other samples, the volumes of solvents, weight of active carbon, etc. are scaled in proportion but the size of the alumina column has been kept constant for 500–900 g of grass. Larger quantities of grass have been processed on this basis and the final concentrates mixed. Equally toxic concentrates have also been prepared to the 3000 level from 3 kg of similar grass, using a large metal extractor of the same type as that described in (1), and processing in one batch apart from step (6) which was done in three batches.

1. Nine hundred grammes of grass is ground finely immediately before it is to be extracted, and put in extractors of the type described by Letham *et al.* (1957), each extractor holding at the most 600 g of grass. The grass is extracted for 8 hours one day and 4 hours the next day, with ether de-peroxided with acidified ferrous sulphate and distilled.
2. The ether extract is put in a large evaporation dish and ether blown off in an air duct when the temperature falls to below 0°c. At the later stage (3 hours) the residue is stirred at times, and the dry residue is stored overnight at below 0°c, protected by a polythene bag.
3. The black tarry residue is rubbed up with 230 ml of absolute methanol for 15 min. The solution is poured off and the extraction repeated with 3 more portions each of 120 ml of methanol. Addition of a wad of cotton wool helps to break up the insoluble part which is hard and waxy at the end. On a smaller scale it is easier to add a little filter-aid to the ether extract, and to remove the methanol-insoluble part by filtration or centrifugation; this does not affect the toxicity.
4. The methanol solution is filtered if necessary and to it is added 27 g of "Darco G-60" activated carbon, and the suspension is shaken at intervals for two hours. It is then filtered, the carbon on the paper washed with up to a further 75 ml of methanol and some solution squeezed out gently.
5. The solution is made up to 600 ml and placed in a separating funnel to which is added 450 ml of b.p. 40–70°c petroleum ether and 150 ml of water. After shaking, the petroleum ether layer is discarded and the methanol-water phase extracted with four more lots each of 400 ml of petroleum ether. Equally good results are obtained using a large liquid-liquid extractor.
6. The methanol phase is usually stood overnight below 0°c, but remains unaltered in toxicity for at least a week. It is put in a one-litre flask of a rotary evaporator under the high vacuum provided by a Stokes Microvac Pump, with water circulating in the separator. The flask is rotated in a shallow dish of water and is allowed to "ice-up". After about 5 hours the methanol has evaporated and the contents of the flasks are transferred to a separating funnel with ether and the aqueous part extracted with three more small volumes of ether. Addition of a little common salt will break emulsions. The ether phase is put in a small dish and evaporated in a powerful air-blast until only a little water remains. A dry residue is obtained by standing for 12–18 hours in a vacuum desiccator over concentrated sulphuric acid at below 5 mm pressure. It is advisable to stir the dried residue and evacuate for a short time to remove occluded water. The residue is often transferred

before it is quite dry to a crystallising dish using acetone, the acetone removed by the air-blast (hair dryer) and the contents dried in the desiccator, to constant weight.

7. An alumina column 10 in. long and 1 in. diameter is wet-packed with B.D.H. "Aluminium Oxide for Chromatographic Adsorption Analysis" or with recovered alumina of activity between I and II, using ether dried by calcium chloride and distilled or "Analar" ether. The ether-alumina slurry is put in the tube from a polythene wash bottle and the alumina tapped down. It is then washed with 200 ml of 2% glacial acetic acid in ether and with 300 ml of dried ether. The solid from step (6) is dissolved in the minimum volume of ether (about 15 ml), a little sodium sulphate added, and put on the alumina, and elution with ether continued until 200 ml of eluate has been collected. This is evaporated in a small dish using the air-blast (hair dryer and fan), and left overnight in a vacuum desiccator as used in step (6) until dry. The residue is an orange gum containing yellow pigments, and usually a trace of porphyrin, or blue pigment derived from the carotenoids. Concentrates at this stage can be kept without loss of toxicity for up to 3 months in the dark *in vacuo,* or in the dark at −10°c, but lose some toxicity when kept in the light *in vacuo,* and become non-toxic on standing in the air and light.
8. The residue is dissolved in a minimum volume of benzene and put on a 6 × 0.5 in. column of activity V alumina, wet-packed with benzene. Activity V alumina is prepared by placing alumina of activity I–II in a vacuum desiccator with 13% of its weight of water in a dish and turning the alumina at intervals until the water disappears. Elution is continued with benzene. The eluate is discarded until the lower part of the leading orange zone has left the column. The fraction collected is the top of the leading orange zone, the dark zone at the top of this, and the small gap before a faint yellow zone. The eluate is typically 70–140 ml. The benzene is evaporated in a beaker in an ice-bath using the air-blast, and the water removed by vacuum desiccation. The residue is a thick yellow-orange syrup.
9. The residue which must not exceed 55 mg is dissolved in 2.5 ml of "AnalaR" carbon disulphide and put on a 9 × 0.4 in. column of magnesium carbonate (B.D.H. "For Chromatographic Adsorption Analysis") / Celite 545 (1 : 1, each dried at 140°c). The column is compacted by pressure. Elution is continued with carbon disulphide, and the leading dark zone (6 ml) containing most of the pigment and weight is discarded. Addition of ether causes a small yellow zone to move down and when this has emerged (e.g. 15 ml), the ether eluate is cold-evaporated and dried. The residue is a slightly yellowish-brown gum, weighing from 9 to 18 mg.

The fractionation procedure is summarised in the following scheme:

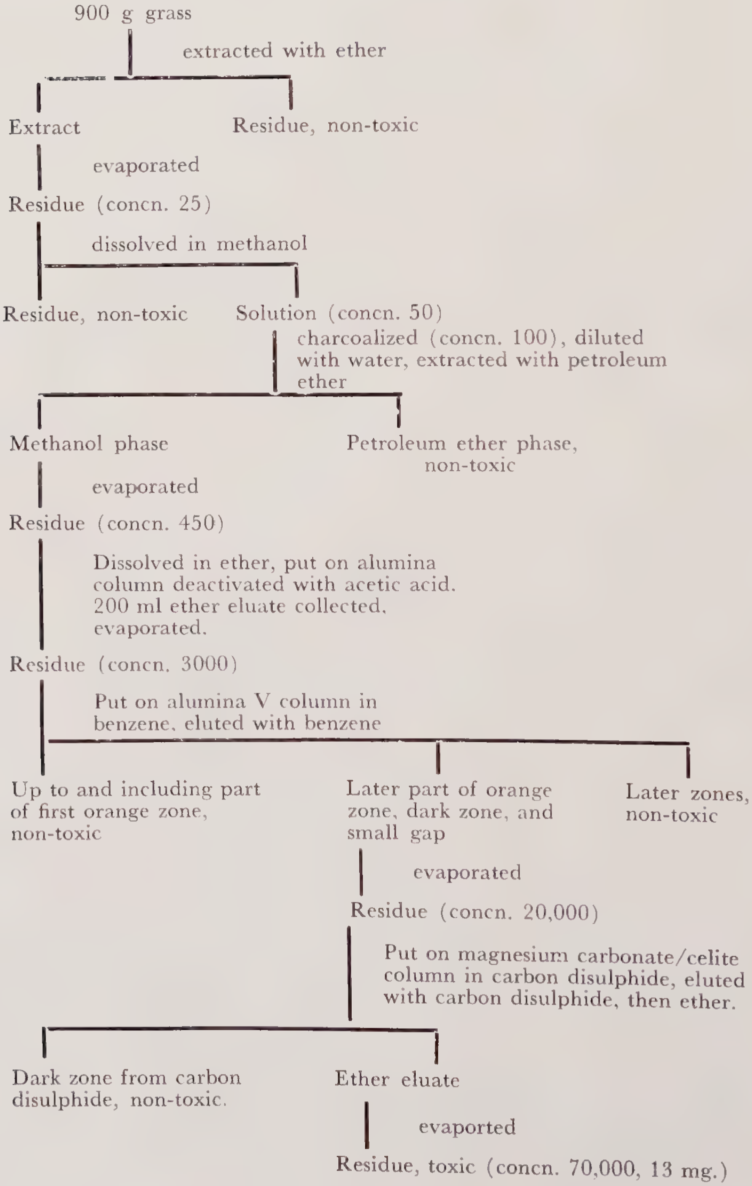
Discussion of Extraction Procedure

The methods adopted have been checked repeatedly on several toxic grass samples. Loss of poison to the non-toxic fractions has been

SCHEME OF FRACTIONATION

Concentrations referred to in this paper are the ratio:

$$\frac{\text{weight grass taken (air-dry weight)}}{\text{weight extract (dried)}}$$


900 g grass
extracted with ether
Extract
Residue, non-toxic
evaporated
Residue (concn. 25)
dissolved in methanol
Residue, non-toxic
Solution (concn. 50)
charcoalized (concn. 100), diluted with water, extracted with petroleum ether
Methanol phase
Petroleum ether phase, non-toxic
evaporated
Residue (concn. 450)
Dissolved in ether, put on alumina column deactivated with acetic acid. 200 ml ether eluate collected, evaporated.
Residue (concn. 3000)
Put on alumina V column in benzene, eluted with benzene
Up to and including part of first orange zone, non-toxic
Later part of orange zone, dark zone, and small gap
Later zones, non-toxic
evaporated
Residue (concn. 20,000)
Put on magnesium carbonate/celite column in carbon disulphide, eluted with carbon disulphide, then ether.
Dark zone from carbon disulphide, non-toxic.
Ether eluate
evaported
Residue, toxic (concn. 70,000, 13 mg.)

remarkably slight, and all such fractions have been fed repeatedly at the usual level, and at levels corresponding to about three times the amount of grass used, without toxicity being detected in them. The substantially quantitative nature of the recovery of poison at each step has been checked repeatedly by dispersal of solutions on grass. Comments on the units of the previous section are as follows:

(1) Batch extractions or percolation with cold or warm ether has shown that even large volumes of this solvent extract less than half of the poison. Continuous methods are necessary. Continuous extraction with acetone is effective in removing the poison, and in concentrates the poison is readily soluble in organic solvents. The extracted grass has been fed for a long time, fortified with ether extract of non-toxic grass without showing toxicity.

The use of copper or stainless steel gauze, or aluminium or tin foil packing in the extractors did not alter toxicity of extracts, and a tinned copper extractor has been used successfully.

(2) For the later stages of concentration of ether or other solutions, the air-blast technique, keeping the solution at about 0°c, leads to no loss of poison, ordinary techniques destroying it. The dried extract can be taken up in ether and a non-toxic "wax" fraction, constituting about a quarter of the weight, centrifuged off.

(3) The methanol-insoluble part contains waxes and much pigment.

(4) The amount of this particularly active carbon added is adequate to remove the bulk of the pigment and to leave a solution comparatively light in colour and containing some carotenoids and porphyrin. Complete removal of colour by carbon has never left a toxic solution, nor has the poison been extractable from the carbon or the carbon found toxic when fed. Evidence on further use of carbon as columns for chromatography is presented later and supports the undesirability of more complete removal of colour at this stage.

(5) When 95% methanol was used, up to a third of the poison was found in the petroleum ether phase (using 3 lots of 2/3 volumes of petroleum ether). Carotene, some porphyrins, and glycerides are removed by the petroleum ether. Dried extracts at various stages taken up in various volumes and boiling points of petroleum ethers, showed that with practical volumes the poison was split between soluble and insoluble fractions, and the best concentrates are characterised by low solubility in this solvent. The methanol phase contains much yellow pigment, with usually a little porphyrin.

(6) Equally good recovery of poison resulted from rotary evaporation to dryness, a slow process, or rotary evaporation with the bath held at 16°c. A more tedious method is to use the air blast with ice-cooling of the evaporating dish, until the temperature cannot be kept below 6°c, followed by vacuum desiccation taking about 36 hours. Satisfactory extraction can be made from 80% methanol using benzene, or from 60% methanol with chloroform, followed by cold-evaporation of these solvents.

In one case, cold-evaporation was taken to a stage where apparently only water remained. The bulk of the poison was in the gum which separated, but some was found in the aqueous part. This may indicate a limited solubility of the poison in water.

(7) In the first trials of chromatography on alumina of activity I–II (Brockmann scale—Brockmann and Schodder 1941), a considerable portion of the poison was eluted by ether/acetone mixtures (concentrations 2000, or up to 4000 in finer fractions), and none obtained by subsequent methanol elution. Subsequent experience showed that ether, acetone, or ether/acetone eluates and fractions of these were generally non-poisonous.

A large number of modifications were tried, designed to find the factor responsible for the loss of toxicity. The modifications included running the column under reducing conditions (stannous chloride alumina (Kofler 1947), hydrogen sulphide, pyrogallol), at lower or higher temperatures, (0 to + 2°c, 27°c), in darkness, with various solvents (dry ether, wet ether, ether/methanol, petroleum ether/acetone), the use of shorter columns (5 in. long), and of neutral alumina of activity I (Lederer and Lederer 1954; Stoll 1947). No improvement was obtained with these changes. A small concentration (× 3) was achieved with a short column and methanol as eluant.

Use of de-activated alumina. A column was washed with acetic acid in ether thus reducing the activity to about V and also neutralising *in situ*. Alumina was also de-activated to activities II, III, IV, and V on the Brockmann scale, by absorption of known weights of moisture from water in a dish, the alumina being spread out in a vacuum desiccator.

The acetic acid washing experiment gave a severely damaged liver and a concentration, using ether elution, of 3500 times. Activities II and III still gave no toxicity in ether-acetone eluates, but activity IV gave a moderate liver damage, and activity V a severe liver damage. Thus it would appear that alumina as formerly used was of too high an adsorptive activity and that provided alumina of activity about V is used destruction of poison does not take place. There are many examples in the literature of changes in substances brought about by high-activity alumina.

Concentrations achieved using ether containing 25% acetone elution were, with activity V, 2660; with activity IV, 3400; with activity III, 3400; and with activity II–I, 4000; compared with the average figure for the acetic-washed alumina using ether alone of 3000. The important point is that lowered-activity alumina still allows a good concentration.

The acetic acid washing technique has been adopted, and the reliability of the procedure shown conclusively by the large number of severely damaged livers found in fractionation experiments including this stage. Unfortunately, with de-activated alumina and ether elution, resolution into defined zones is not clear, and there is no potentiality of collecting finer fractions. The poison is eluted near the front of the yellow area on the column, and 200 ml of eluate leaves a margin of safety.

(8) The poison has not been detected in the colourless and later yellow eluate before the first major orange zone, or in the lower part of the orange zone, but a trace was detected in the small gap just above the dark zone. There was none in the small yellow zone immediately following the gap. It is not normally practicable to cut the zones collected more finely.

Many modifications tried, including larger columns, use of alumina containing 16% or 20% water, use of alumina de-activated by acetic acid in benzene sufficiently to stop destruction of the poison, or the use of petroleum ether/benzene mixtures gave good recovery of poison in certain zones, but resolution into zones was not so clear, and lower weight concentrations were obtained.

There is a persistent small loss of poison which is not avoided by running the whole operation in the dark. When the routine step was repeated to give finer fractions, no poison was recoverable.

(9) When the weight of residue from the previous stage on occasions exceeded 55 mg, the poison usually appeared entirely in the dark zone, and close to it, giving little concentration. Many variations of conditions showed the details given to be the best, and a likely procedure for the larger weights is to use two similar columnns each loaded with half the extract. The poison appears in the later part of the eluate near the small yellow zone which forms near the top of the column. Finer cutting of the eluate did not always confine the poison to a defined area of the column.

OTHER INFORMATION ABOUT THE POISON

Effect of Acid and Alkali

Ether solutions of concentrates were used to secure information on basic or acidic properties of the poison. The toxicity remained in the ether phase after shaking with several small volumes of approximately 1% aqueous HC1, or with 2.5% Na_2CO_3, or 1% $NaHCO_3$ in water. The aqueous part was used as a mixing water for non-toxic grass in feeding to guinea pigs. Extractions with 20–60% hydrochloric acid to extract porphyrins gave no recoverable toxicity. The concentrate at 20,000 times when treated with a minimum quantity of 85% sulphuric acid to remove carotenoids (Parker and McFarlane 1940) left no toxicity in the benzene.

Ether solutions were also extracted rapidly with 5% aqueous sodium hydroxide or shaken with aqueous sodium hydroxide for 24 hours to give a "cold" saponification, in one experiment with pyrogallol present to prevent autoxidation (as used for tocopherols—Tosic and Moore 1945). Normal saponification was also done. None of the various fractions recovered from these experiments was toxic.

Use of Carbon and Carbon-column Chromatography

The addition of carbon to give complete decolourisation of purified concentrates in methanol has not left a toxic solution. Concentrates at various stages were put on Darco-Celite columns in methanol, and eluted with methanol, benzene, and quaternary detergents, and in some cases a reducing atmosphere was provided by hydrogen sulphide. Low levels of poison have at times been recovered in methanol or benzene eluates, and the residual carbon has been fed direct, or Soxhlet extracts made with many solvents without recovering the poison.

Use of Other Chromatographic Columns

Using ether as solvent and eluant after the 450 stage, other chromatographic media were used, e.g. magnesia/Celite, calcium carbonate, silica gel, magnesium silicate, giving good recovery of poison with little concentration.

After the 3000 stage other media were tried as an alternative to alumina V, using benzene as solvent, and this with more polar solvents as eluants. Concentrations of about 8000 times were obtained from benzene/ether fractions using Florex XXX/Celite, Floridin/Celite, Magnesol/Celite, Florisil, Silene EF/Celite, and silica gel, usually with some loss of poison and zones were not easily defined. Floridin had the best potentiality.

After the 20,000 stage were tried magnesium carbonate/Celite with carbon tetrachloride, alumina containing 13–20% water with carbon disulphide/benzene, magnesium oxide, calcium hydroxide/Celite, and silica gel with carbon disulphide/ether. No poison was obtained in total eluates or fractions.

Paper Chromatograms

Using the carbon tetrachloride/methanol/water system described in the next section, and the whole of a 20,000 concentrate put on a large Whatman No. 3 disc, as a two-inch-diameter ring using an air blast, the poison was recovered from the main yellow zone near the solvent front, with little concentration. Cellulose powder columns done in various ways using this solvent system have at times shown poor recovery of poison, usually none. It has not been possible to recover poison from the ethyl oleate system.

Counter-current Distributions

Counter-current distributions using 30 small separating funnels as tubes have been tried after the 3000 stage. The solvents were aqueous methanol and various non-polar solvents, and were:

(1) Carbon tetrachloride/methanol/water, 62:35:3.15 as used by Cole *et al.* (1953) for lipids,

(2) Petroleum ether b.p. 40–60°c / benzene / methanol / water, 65:35:90:10.

(3) Chloroform/methanol/water, 50:40:15.5 (all ratios are volume measurements).

Each distribution used 15 ml of each phase and double the usual feeding level of the 3000 concentrate. Each fraction was cold-evaporated and weighed, and beakers grouped to give 6 fractions for feeding. Except for the carbon tetrachloride system which showed more spread, and may have given several peaks if many more tubes had been used, the bulk of the weight was well defined in one peak. Poison was recovered in each case but was on the peak of weight, so that little concentration was achieved.

A 10-tube counter-current using carbon disulphide/methanol/water 50:45:5 was done after the 20,000 stage. The poison was in the first tubes containing most of the weight, but most of the colour moved with the carbon disulphide phase. It is concluded from these distributions, and from paper work, that the bulk of the components and the poison have very similar partition coefficients in the above solvent systems, and that many tubes would be needed to give a separation.

COMPARISON OF CORRESPONDING FRACTIONS FROM TOXIC AND NON-TOXIC GRASS

Comparisons have been made between fractions known to be poisonous, and similar fractions from non-toxic grass, in the hope of finding obvious differences that might be associated with toxicity.

Perrin (pers. comm.) compared weights, and absorption spectra in the visible and ultra-violet, of concentrates at the stages up to 3000 times, also fractions from high- and low-activity alumina using ether, and ether/acetone, without finding pronounced differences. Similar work on fractions obtained during development of concentration techniques led to the same conclusions. This work showed the presence of substance m.p. 260°c, in toxic samples but not in non-toxic samples, and this substance, which is not the poison, has provided the basis for the "beaker test". (See Part XIII).

The ultra-violet spectra of toxic and non-toxic concentrates at the later stages, of fractions of each, of the 70,000 concentrate, and of fractions beyond this, have also failed to produce any difference consistently associated with toxicity. The best concentrates show very little absorption in the ultra-violet.

At the "3000" stage the concentrations achieved with routine runs, using 7 grass samples of different toxicities and 3 non-toxic samples were in the range 2000 to 6500 (total 57 runs), and with the same samples at "20,000", 16,000 to 45,000 (22 runs). The next step also produced concentrations in the range 50,000 to 120,000 from toxic or non-toxic grasses. The higher concentrations were not always from non-toxic samples. No weight differences were found in fractions beyond 70,000.

Paper chromatograms using the disc technique (White 1957) have been investigated to compare toxic and non-toxic fractions. The main guide has been ability to resolve the yellow pigments or substances fluorescent under ultra-violet light. With many conventional systems or with papers impregnated with Vaseline, paraffin oil, or silicones, or with acetylated papers, the yellow pigments have remained together at the origin or at the solvent front, or have tailed badly without showing worthwhile separation.

Olive-oil impregnated paper with aqueous alcohols as developing solvent has been used to resolve carotenoids (Nunez 1954). Papers impregnated with vegetable oils, or preferably ethyl oleate, or glycol stearate, "Quilon", rubber solution, or rubber latex in conjunction with 80% or 90% methanol have shown the presence of several yellow or fluorescent components, but none have revealed distinctive differences between extracts of toxic or non-toxic grasses at the 3000 or 20,000 stages.

The solvent system carbon tetrachloride/methanol/water 50:41:9, the bottom phase being put in the desiccator and the top phase as developing solvent, gave good resolution of yellow pigments on unimpregnated paper. At the 20,000 stage this system gave 3 main yellow zones, and 2 minor incompletely resolved ones. The number, position, and appearance of the zones were the same for toxic and non-toxic preparations. These systems do not give clear resolutions of pigment zones at the 70,000 stage and beyond.

So far nothing has been detected on papers by staining with reagents, except on the yellow zones. Oxidising agents, particularly aqueous or acetone permanganate, or chromic acid in acetic acid, show stained areas due to yellow pigments or to high enough concentration of substances m.p. 149° or 260°C.

DISCUSSION

The results presented are from about 240 fractionation experiments using 700 guinea pigs.

The lesions obtained in guinea pigs by feeding extracts are macroscopically and histologically identical with those produced by feeding the dried grass. These are compatible with lesions in lambs fed the same dried grass and in sheep grazing toxic pasture (Evans *et al.* 1957). The type of liver damage is not known to be produced by any chemical from synthetic or plant sources, fed at dose levels comparable with those of the facial-eczema poison.

The fractionation procedure to give concentrations of 50,000 to 100,000 times has been checked many times. About half the poison has been recovered, the loss occurring in the alumina V step, and no better alternative has been found. Concentrates at the 20,000 level have been made dozens of times using several toxic grass samples.

Occasional preparations at any stage do not give liver damage, but whether this is due to destruction in manipulation, or to guinea pig variation is not known. The evidence from this work and from that of Perrin (1957) is that guinea pig variation is not serious at the high level of poison being used.

The poison is stable in methanol solution for at least some days, and dried concentrates at the 3000 level can be kept for months in the dark *in vacuo* or at low temperature. The main instability occurs in the later stages of concentration of solutions, and special techniques were devised to overcome this. There is also ready destruction of the poison by high-activity alumina or much active carbon. Considerable manipulation of solutions after the 3000 stage is possible without any evidence of instability due to air or light, and at no stage has the provision of reducing atmosphere prevented loss of poison. Beyond the 20,000 stage many seemingly mild techniques lead to complete loss of poison, but some methods using carbon disulphide give a good recovery.

The poison, at least in highly purified concentrates, is readily soluble in ether, acetone, benzene, methanol, 80% methanol, chloroform, and carbon disulphide, fairly insoluble in petroleum ether and water.

Results of acid and alkaline extractions from organic solvents show that it is not a base or typical alkaloid, or free organic acid, and is neutral. Concentrated acids destroy it. It is quickly destroyed by dilute sodium hydroxide, and, if an ester or glyceride-like substance or lactone, it is one insoluble in petroleum ether. Tests for esters or lactones in concentrates have been negative. Large numbers of typical types of naturally occurring organic compounds are ruled out, for example it is not a typical protein, peptide, phosphatide, or porphyrin, and is unlikely to contain aromatic nuclei. Traces of yellowish pigment, possibly carotenoid, but not flavonoid, are present in the best concentrates, and no toxic preparation has been colourless.

The properties are consistent with those of a neutral organic compound, with little if any colour, of medium molecular weight, and devoid of characteristic absorption in the ultra-violet. It is likely to be almost saturated, and to contain alcoholic or similar uncharacteristic oxygen functions to account for low solubility in petroleum ether.

Corresponding fractions from toxic and non-toxic grasses show a remarkable similarity in weight, absorption spectra, the nature of pigments, and other properties, even at the 70,000 level and beyond. If the poison has characteristic chemical and physical properties it must be present in the 70,000 concentrate in minute concentration. There is the possibility that an optical difference could account for toxicity.

A substance of m.p. 149°c has been isolated from the 3000 stage concentrates of toxic and non-toxic grasses. A second substance of m.p. 260°c has been obtained from similar concentrates and is generally found in toxic samples only. The isolation and characterisation of these substances will be described in a subsequent paper (Part XIII). These substances are close to the poison on chromatographic columns but there is no evidence that the poison is related chemically to them, and data from feeding experiments, particularly the fact that they have been largely removed from the more refined concentrates, indicates that they are not the poison. Substances capable of drastic hydrolysis to amino acids, as is substance m.p. 260°c, are present in both toxic and non-toxic concentrates at the 70,000 level, but in very low concentration. At least five amino acids could be found in such hydrolysates, with no obvious difference associated with toxicity. If the poison is allied to the substance m.p. 260°c, it must be in the best concentrates in very low concentration.

Work on fractionation after the 70,000 stage is continuing along the major lines of finer adsorption chromatography, disc-paper chromatography, cellulose powder column chromatography and counter-current distribution. So far a further routine concentration step has not been made, but on two occasions recoveries of about half the poison of the original grass have been obtained at concentrations of 300,000 and 450,000 times. This means that from 900 g of highly toxic grass half the poison has been obtained in two to three milligrammes of faintly yellow-coloured gum.

The poison is present in very low concentration (on recent evidence below 5 p.p.m.) in dried pasture that produces severe liver damage in guinea pigs. The concentration in grasses in which lower levels of toxicity are detected by high-level guinea pig feeding must be much less. It must be a substance of very potent biological activity, and of a highly specific nature.

ACKNOWLEDGEMENTS

Throughout this work considerable discussion and helpful suggestions have come from Mr. N. T. Clare and Dr. D. D. Perrin. Certain observations quoted in the paper were made by Dr. Perrin, particularly some absorption spectra studies, and the recognition of the presence of substance m.p. 260°c on alumina columns.

Mrs. Jean Sandos carried out experiments on extracting the poison with ether by batch and percolation methods.

I have to thank Mr. N. M. Lauriston for preparing and recovering the large volumes of solvents used, and various people for routine feeding and care of guinea pigs, particularly Miss G. J. Falconer and Miss G. Hughes.

REFERENCES

BROCKMANN, H.; SCHODDER, H. 1941: *Ber. dtsch. chem. Ges. 74B:* 73.

CLARE, N. T. 1952: "Photosensitization in Diseases of Domestic Animals" p. 24. Commonw. Agric. Bur., Farnham Royal.

——— ——1955: "Advances in Veterinary Science", Vol. 2 pp. 182-211. Academic Press, New York.

COLE, P. G.; LATHE, G. H.; RUTHVEN, C. R. J. 1953: *Biochem. J. 54:* 449.

EVANS, J. V.; MCFARLANE, D.; REID, C. S. W.; PERRIN, D. D. 1957: *N.Z. J. Sci. Tech. A38:* 491, 680.

FILMER, J. F. 1951: *Proc. Specialist Conf. Agric., Adelaide,* p. 320. H. M. S.O., London.

KOFLER, M. 1947: *Helv. chim. Acta 30:* 1053.

LEDERER, E.; LEDERER, M. 1954: "Chromatography" p. 21. Elsivier, Amsterdam.

LETHAM, D. S.; PERRIN, D. D.; RONALDSON, J. W.; WHITE, E. P. 1957: *N.Z. J. Sci. Tech. B38:* 695-6.

NUNEZ, G. 1954: *Bull. Soc. Chim. biol., Paris 36:* 411.

PARKER, W. E.; MCFARLANE, W. D. 1940: *Canad. J. Res. B18:* 405.

PERRIN, D. D. 1957: *N.Z. J. Sci. Tech. A38:* 669.

PERRIN, D. D.; WHITE, E. P.; CLARE, N. T. 1953: *Proc. N.Z. Soc. Anim. Prod. 12:* 121.

SIMPSON, J. E. V.; SINCLAIR, D. P.; SWAN, J. B.; FILMER, J. F. 1957: *N.Z. J. Sci. Tech. A38:* 947.

STOLL, M. 1947: *Helv. chim. Acta 30:* 991.

TOSIC, J.; MOORE, T. 1945: *Biochem. J. 39:* 498.

WHITE, E. P. 1957: *N.Z. J. Sci. Tech. B38:* 707-9.

MOLYBDENUM IN RELATION TO THE OCCURRENCE OF XANTHIN CALCULI IN SHEEP

By H. O. Askew, Cawthron Institute, Nelson

(*Received for publication, 3 March 1958*)

Summary

Samples of untreated Moutere Hills pastures contained from 0.03 p.p.m. down to unmeasurably small amounts of molybdenum in the dry matter, whereas pastures from other formations gave figures ranging from undeterminable amounts up to 0.4 p.p.m. Xanthin calculi have been found in Nelson only on the Moutere Hills formation.

Gorse tips eaten by sheep may contain more or no more molybdenum than pastures on the same area, according to location.

Copper contents of pastures and gorse tips were normal to high.

Sheep livers from healthy Nelson pastures were normal in molybdenum content. Livers from a field trial on an affected farm contained from 0.7 to 2.6 p.p.m. Mo; high molybdenum content was correlated with lessened incidence of calculus trouble. Down to 0.8 p.p.m. Mo has been found in other Nelson areas without occurrence of calculi.

Protein intake may be an important factor governing incidence of calculi.

Circumstantial evidence points to low molybdenum intake as a predisposing cause of calculus formation in sheep.

Introduction

The occurrence of xanthin calculi in sheep grazing pastures on the Moutere Hills of Nelson has been described previously (Easterfield, Rigg, Askew, and Bruce 1929; Easterfield and Bruce 1930). It was pointed out there, and in another publication dealing with Moutere Hills pastures (Rigg and Askew 1930), that the pastures are of poor feeding quality, especially during the summer. This applies particularly to their phosphate and protein contents.

Later work (unpublished) by the Cawthron Institute showed that great improvement in stock health and in carrying capacity took place when the land was treated with lime and phosphate, together with the sowing of clovers; provision of extra winter feed was valuable. A conclusion from this work was that an economic cure for the unsatisfactory results with stock on this type of hill country had been obtained. But the true cause of the development of calculi in the kidneys of stock was not found before work in this field had to be discontinued.

During the above work the tendency was to explain the occurrence of calculi as being due to a deficiency of calcium, phosphorus, protein, vitamins, or of several of these combined. The idea of a mineral deficiency being the cause was thus entertained but that it might be due to a deficiency of one of the trace or micro-nutrient elements was not considered at that time. It was thought however that the conditions met with by the stock had some effect on the activity of

N.Z. J. agric. Res. 1: 447–454.

xanthin oxidase. With the demonstration a few years ago (Westerfeld and Richert 1954) that this oxidase requires the micro-nutrient element molybdenum as an activator the xanthin calculus problem took on a new aspect. The present paper discusses such evidence as is available on correlations between molybdenum content of soils, pastures, and animal organs, and the occurrence of xanthin calculi.

EXPERIMENTAL

The molybdenum contents of soils, pastures, and animal organs have been determined by the thiocyanate method after wet digestion of the ashed material with perchloric, nitric, and sulphuric acids.

Soil

The data of Kidson (1954) show that soils derived from the Moutere Hills formation are low in total and available molybdenum, the former being less than 0.3 p.p.m. and the latter, in the one case examined, being only 0.02 p.p.m. For other soils of the Waimea County, on which xanthin calculi are not found, the total and available molybdenum contents range respectively from 0.4 to 3.2 p.p.m., and from 0.11 to 0.19 p.p.m. It is interesting to find that a soil derived from granite at Kaiteriteri had only 0.2 p.p.m. acid-soluble and 0.03 p.p.m. available molybdenum. Very few stock are to be found in this area, but so far as is known xanthin calculi do not occur. Other soils derived from granite were also found to be very low in molybdenum. A point of interest here is that on the granite formation at Glenhope where numbers of sheep have been grazed for many years xanthin calculi have not been found. It is of interest to note that Kidson reported that the magnesian soils from Mineral Belt material are also very low in molybdenum.

On the Moutere Hills soils very favourable responses to applications of molybdenum to pastures have been obtained. Use of ground limestone also increases the level of molybdenum in the pasture (unpublished experiments). Farmers report too that granite soils are similarly responsive.

Occurrences of xanthin calculi in sheep have been reported from other parts of New Zealand, in both the North Island (Anon., 1933) and the South Island (Dr. C. S. M. Hopkirk, pers. comm.). So far as can be ascertained these occurrences were in stock grazing on soils now known to be responsive to applications of molybdenum, but the locations and their soil types have not been precisely determined.

Pastures

Many poor pastures in which legumes are almost entirely absent are to be found on the Moutere Hills. Young growth after a burn is relatively rich in lime, phosphate, and nitrogen, but older growth, especially in summer, may be very low in these constituents in the absence of fertiliser treatment. Use of ground limestone and phosphatic fertilisers greatly improves the condition and chemical composition of the pasture.

Determinations of molybdenum content of a number of pastures sampled in November 1953, from the Moutere Hills and other formations are given in Table 1. The data are given as parts per million of molybdenum in the dry matter. Copper contents of the pastures and pH values of the soils (0-6 in. depth) on which the pastures were growing are given also.

TABLE 1. MOLYBDENUM AND COPPER CONTENTS (p.p.m. of D.M.) OF PASTURES, AND pH VALUES OF THE SOILS

Location	Remarks	Pasture Mo p.p.m.	Pasture Cu p.p.m.	Soil pH
Dovedale Hill, Top	Very poor growth, fescue	0.03	8.7	5.1
Pigeon Valley, S. side	Mainly fescue, untreated	0.03	9.7	5.1
Pigeon Valley, N. side	Very mossy area, untreated	0.03	11.7	5.2
88 Valley	Untreated	0.02	12.6	4.9
88 Valley	Same location, resown, topdressed	0.03	14.3	—
Trass Valley	Untreated, hillside	<0.02	10.6	5.3
Pigeon Valley	Untreated, poor pasture on hill	<0.02	19.1	5.3
Pigeon Valley	Resown, topdressed	0.14	23.7	—
Eves Valley	Untreated hill	0.02	11.8	5.0
Eves Valley	Resown, topdressed	0.02	12.8	—
Aniseed Hill	Heslington formation	0.38	12.3	—
Lee Valley	Maitai formation	0.11	11.6	5.5
Lee Valley	Mineral Belt formation	<0.02	8.6	7.2

In the Moutere untreated pastures, the main grass species have been fescue, browntop, sweet vernal, and danthonia; legumes were almost completely absent. It is notable too that all the soils on which the pastures were growing had low pH values. Molybdenum contents of untreated pastures have been 0.03 p.p.m. or lower. Copper contents are normal to high so that the question of insufficient supply of copper for the animals does not enter; on the other hand a high copper content in conjunction with a very low molybdenum content may not be without some effect on the animal—a purely conjectural point in the present state of knowledge of relationships between copper and molybdenum.

Where ploughing, fertilising, and resowing have been done, as represented by samples from 88 Valley, Pigeon Valley, and Eves Valley, the molybdenum content of the pasture has not always been increased; the relatively high figure of 0.14 p.p.m. Mo at Pigeon Valley is due to the use of molybdenised superphosphate. All these pastures consisted of good mixtures of English grasses and clovers. Copper contents of these improved pastures are also greater than those of the corresponding untreated ones.

The Mineral Belt (Serpentine formation) is represented by one sample only, which has given a molybdenum figure as low as any pasture of the Moutere group; copper content is normal. Calculus in sheep has not been reported from this formation. Grazing is extensive in this area and animals have access to soils derived from limestone, etc. On the Maitai and Heslington formations the molybdenum contents were relatively high at 0.11 p.p.m. and 0.38 p.p.m. respectively. Copper contents were approximately 12 p.p.m. Both of these formations are healthy to stock.

Gorse Tips

Because farmers have related decreases in the incidence of calculi to invasion of pastures by gorse it was thought necessary to obtain samples for analysis of tip growth of gorse plants, which is freely eaten by sheep. In all cases the samples were from gorse plants growing in the fields from which the pasture samples of Table 1 were collected. Molybdenum and copper contents of these gorse tips are given in Table 2.

TABLE 2. MOLYBDENUM AND COPPER CONTENTS (p.p.m. of D.M.) OF GORSE TIPS, AND pH VALUES OF THE SOILS (0–6 in. depth)

Location	Geological Formation	Gorse tips Mo p.p.m.	Gorse tips Cu p.p.m.	Soil pH
Pigeon Valley, N. side	Moutere Hills	0.09	12.6	5.2
Pigeon Valley	,, ,,	<0.02	14.0	5.3
Trass Valley	,, ,,	<0.02	13.3	5.3
Eves Valley	,, ,,	0.02	13.3	5.0
88 Valley	,, ,,	0.03	14.1	4.9
Aniseed Hill	Heslington	0.87	13.0	—
Lee Valley	Maitai	0.21	13.1	5.5
Lee Valley	Mineral Belt	<0.02	10.6	7.2

For the five samples from the Moutere Hills the molybdenum contents vary from 0.09 p.p.m. to less than 0.02 p.p.m. (the lower limit of estimation by the method of analysis employed). In some cases therefore the molybdenum content of the gorse tips was just as low as that of the pasture. Copper content in each case was comparable to that of the corresponding pasture.

On the Mineral Belt the molybdenum content of the gorse tips was as low as that of the pasture, but on the Maitai and Heslington formations the gorse tips were twice as rich in molybdenum as the corresponding pasture. It would appear therefore that any benefit to the stock on Moutere country from eating gorse tips is not due to enhanced intake of molybdenum.

But points to be considered relate to the phosphoric acid and nitrogen contents of gorse tips compared with pastures on the Moutere Hills. Data for the samples used for the molybdenum determinations are not available, but data were obtained a number of years ago for samples from a number of areas (unpublished data of Cawthron Institute). In Table 3 are set out data for the period December to

May for untreated and treated pastures on a farm where calculus trouble was serious: the treated pasture received 18 cwt ground limestone and 4 cwt basic slag per acre. In this table also are given average figures for good Nelson pastures and the ranges of values found for gorse tips from different parts of the Moutere Hills.

TABLE 3. PHOSPHORIC ACID AND NITROGEN CONTENTS OF MOUTERE HILLS PASTURES, GOOD NELSON PASTURES, AND GORSE TIPS (% of D.M.).

Constituent	Moutere Hills		Good Nelson Pastures (average)	Gorse Tips
	Untreated	Treated		
Phosphoric acid (P_2O_5)	0.26–0.45	0.34–0.55	1.06	0.36–0.79
Total nitrogen (N)	1.01–1.92	1.19–2.00	5.34	2.72–3.61

The data of Table 3 show that gorse tips will probably have a higher content of phosphoric acid than a moderately treated pasture (not resown), and that they will certainly be much richer in nitrogen than that pasture. The gorse tips are not however equivalent to a good pasture in supplying phosphoric acid and nitrogen. It is important to note that although severe xanthin-calculus trouble occurred on the untreated pasture of this Moutere Hills farm, it was much reduced on the treated pasture (unpublished field experiments discussed in the next section of this paper).

Animal Organs

According to available figures the normal molybdenum content of sheep's liver ranges from 2 to 6 p.p.m. in the dry matter (Dr. I. J.

TABLE 4. MOLYBDENUM CONTENT OF NELSON SHEEP LIVERS (p.p.m. of D.M.).

Origin	Molybdenum (Mo) p.p.m.
Cawthron Institute (Brook st. Igneous)	5.6
Cawthron Institute (Brook st. Igneous)	4.6
Pigeon Valley and Stoke	3.9
Appleby	3.3
Takaka	3.2
Glenhope (granite)	1.2
Glenhope (granite)	1.1
Sherry River (granite)	0.9
Sherry River (granite)	0.8
Pigeon Valley:	
Phosphate and protein lick for stock	2.6
Limestone and phosphate on pasture	2.0
Limestone on pasture	1.7
Superphosphate on pasture	1.2
Untreated pasture	0.7

Cunningham, pers. comm.). Data for Nelson sheep's livers from healthy non-Moutere Hills country and from a Moutere Hills field trial are given in Table 4.

The above figures show that for Nelson sheep grazed on healthy, non-granite, country the molybdenum content of the liver is quite within the range of published normal data, but that livers from sheep grazing granite country, on which cobalt deficiency of stock is found, contain only about one part per million of molybdenum. No calculus has, to the author's knowledge, ever been found in sheep at Glenhope or Sherry River. On the other hand it is known that pastures on these granite soils respond to molybdenum topdressing, even though the molybdenum content of the untreated pasture is normal.

In the lower portion of Table 4, data are given for five samples from Pigeon Valley. These represent animals in a field trial in the 1929-30 season under the control of Mr. (later Sir) Theodore Rigg. The farm where the trial was carried out had a record of high incidence of calculi in the kidneys of sheep. Because only small numbers of sheep were available for each treatment (owing to the low carrying capacity of the pasture) the real value of the treatments cannot be strictly evaluated, but the facts recorded were that with the mineral and protein lick, with lime and phosphate treatment of the pasture, and with the use of ground limestone on the pasture no deaths of animals occurred, but one animal on the first treatment showed symptoms of calculus trouble. On the area receiving superphosphate one animal died and on the untreated area several animals died, and another was affected by calculus trouble. All of these deaths were directly attributable to calculi in the kidneys. Where the molybdenum content of the dry matter of the liver was approximately 2 p.p.m. (and this is in the lower part of the range of published normal figures) calculus formation probably did not occur, but at lower levels of molybdenum calculus formation took place. The chemical data therefore correlate with the field observations of this experiment.

DISCUSSION

From farmers' reports, sheep could mostly be carried satisfactorily for some years after clearing bush or scrub from the Moutere Hills, but in the areas where in later years the occurrence of xanthin calculi became a problem it is stated that calculi were found in sheep in the early 1890s. The trouble is not usually found in lambs but is common in hoggets and older sheep, especially in wethers. Sheep bred on an unhealthy property are more severely and more quickly affected than animals brought in. All varieties of sheep appear to be susceptible to the ailment. Calculi are more likely to appear following dry seasons. In more recent years, owing to invasion of pastures by gorse say some farmers, and probably owing to better pastures and to supplementary feed, incidence of calculi is lower than previously. As has been stated earlier, occurrence of xanthin calculi in the Nelson area is confined to the Moutere Hills formation and in severe form to a relatively restricted area on the Rosedale silt loam and Spooner stony silt loam phases of that formation.

Now the fact that calculus trouble is worse after dry weather could be a result of decreased protein, and vitamin contents, and decreased content or availability of minerals such as phosphate and molybdenum. Analytical data already presented show that the protein and phosphate contents of untreated Moutere Hills pastures are low. Work overseas (Ferguson, Lewis, and Watson, 1943) has shown that when pasture plants are dried the availability of molybdenum to stock is reduced. In a dry summer therefore the amount of molybdenum, already low, in the pasture would be reduced, possibly to an undesirably low level. It is true that the fact that pastures on areas other than the Moutere Hills which contain no more molybdenum than those pastures, and that livers of animals depastured on granite country may be as low in molybdenum as those of animals affected by calculi, but do not lead to the development of calculi in the animals, appears to negative the idea that a deficiency of molybdenum is the cause of the formation of xanthin calculi in sheep.

There is however a further matter of which cognisance must be taken, namely the apparent direct relationship discovered by Westerfeld and Richert (1954) between protein level of the diet and molybdenum requirement for establishing a satisfactory level of xanthin oxidase in the rat. There is no doubt whatever that on topdressed and improved pastures on the Moutere Hills, on pastures on other formations, e.g. granite country, xanthin calculi are not known, or where the stock have access to gorse tips and possibly other herbage (even weeds), the protein content of the ingested material will be markedly higher than on untreated Moutere Hills country. Thus, by analogy with the results obtained with rats, it would be expected, that, if deposition of xanthin is due to an abnormally low level of xanthin oxidase in the animal, calculus formation would be reduced or even completely suppressed. Westerfeld and Richert (1954), however, state that all species do not have the same xanthin oxidase. On the other hand the effect of the known reduction in protein level of pastures in dry weather, and of accompanying reduced availability of molybdenum is in keeping with farmers' observations that incidence of xanthin calculi is much increased by the end of a dry season. That some stored molybdenum may be used up by animals from healthy country is shown by the buying-in of such animals by farmers on affected country: such animals do much better and are not affected by calculi as early as animals bred on unhealthy country. The non-occurrence of xanthin calculi in sheep grazing resown and topdressed pastures could be due to the combined influence of increased level of molybdenum and nitrogen (and other elements?) in the pasture.

That the molybdenum status of the food of the animal has some influence on the occurrence of xanthin calculi in sheep appears to be reasonably certain, because not only in Nelson but also in North Auckland and in Otago the soils producing unhealthy pastures are in molybdenum-deficiency areas. How far other factors such as copper, manganese, phosphorus, sulphur or protein content of the foodstuff also enter is not clear. Moreover there is no definite evidence of what other function, besides acting as activator to xanthin oxidase, molybdenum may have in animals.

A reasonable conclusion seems to be that low molybdenum content of the feed is a factor causing the appearance of xanthin calculi in stock. At present, in the absence of controlled feeding, the evidence is circumstantial but consistent.

ACKNOWLEDGEMENT

The author wishes to thank Miss Joyce Watson for determining the molybdenum and copper contents of the samples discussed in this paper.

REFERENCES

ANON. 1933: *N.Z. J. Agric. 47:* 404.
EASTERFIELD, T. H.; BRUCE, J. A. 1930: *N.Z. J. Sci. Tech. 11:* 357.
EASTERFIELD, T. H.; RIGG, T.; ASKEW, H. O.; BRUCE, J. A. 1929: *J. agric. Sci. 19:* 573.
FERGUSON, W. S.; LEWIS, A. H.; WATSON, S. J. 1943: *J. agric. Sci. 33:* 44.
KIDSON, E. B. 1954: *N.Z. J. Sci. Tech. A36:* 38.
RIGG, T.; ASKEW, H. O. 1930: Moutere Hills Pastures. *N.Z. Dep. sci. industr. Res. Bull. 19.*
WESTERFELD, W. W.; RICHERT, D. A. 1954: *Ann. N.Y. Acad. Sci. 57:* 896.

THE USE OF CHLORPROMAZINE IN THE MEASUREMENT OF RADIOACTIVE IODINE IN THE THYROID GLAND OF THE RAT

By Evan Wright, Animal Research Station, Department of Agriculture, Wallaceville.

(*Received for publication, 20 February 1958*)

Summary

High levels of chlorpromazine given as a single dose did not affect the absorption of radioiodine by the thyroid gland of the rat, and may be used to control the animals during measurements of radioactivity.

Prolonged daily administration caused a marked reduction in accumulation of radioiodine.

Introduction

When making radioactivity measurements on rats, general anaesthesia is frequently used to control them but, besides the risk of losing the animal, this procedure is not conducive to rapid routine measurement. In addition, some anaesthetics interfere with thyroid function (e.g. thiobarbiturates, Wase and Greenspan, 1953).

To overcome this difficulty Fish, Carlin and Hickey (1952) used a glass cone into which the rats were trained to crawl and in which they remained comfortably for the peroid of counting. When I tried this method with rats from the Wallaceville colony I found that they could be coaxed into the tube once but completely refused to be put in a second time. In case this reaction depended on the material of construction, tubes were made from glass, metal, celluloid, and cardboard, but in every case the same result ensued and in the end the attempt was abandoned. Instead, I used the holder described in the next section. Like the cone, this holder is not readily accepted by the Wallaceville rats and they usually struggle but, in contrast to the cone, they can be easily put, and held, in the holder. Though it is feasible to carry out experiments under these conditions, it is preferable to use some means of quietening the animals.

While looking for such a means my attention was drawn to the tranquilising drugs, in particular chlorpromazine. This drug, whose pharmacological properties have been described by Courvoisier *et al.* (1953) removes fear and produces indifference to discomfort. It has been used extensively, in both human and veterinary medicine, as an enhancer of anaesthetics (e.g. Smith and Fairer 1953; Weaver 1956), for the control of tetanus symptoms (e.g. Kelly and Laurence 1956; van den Heever 1955), and in psychiatry (Lomas 1955). In the field of animal handling, Troughton, Gould, and Anderson (1955) report the use of chlorpromazine in the control of unmanageable domestic animals, while Estrada (1956) has reported its suitability for quietening dogs, and Das *et al.* (1954) found similar advantages in handling vicious monkeys.

N.Z. J. agric. Res. 1: 455–461.

To be useful for the control of rats for the measurement of thyroidal radioactivity, the drug must be without influence on thyroidal function. This paper describes experiments carried out to find any such influence. The experiments are in two groups, the first of which were made with a G.M. Counter over a short period and are subject to considerable experimental error. Two factors were instrumental in the decision to perform the more elaborate experiments of the second group. Firstly, the finding of Mayer, Kelly and Morton (1956) that chlorpromazine was strongly antithyroid when added to the culture media of thyroid slices, and secondly, the acquisition of a scintillation counter which, together with the use of larger numbers of animals, reduced the experimental error.

EXPERIMENTAL METHODS

The Rat Holder

The apparatus used to restrain and hold the rat in position is shown in Fig. 1. It is based on a lead plate 1 inch thick which has a window above the radiation detector. In this plate are four steel posts to prevent horizontal movement of the rat and one larger post on which there moves an arm carrying a pad of sponge rubber 1 inch thick. This arm is raised above the positioning pins and rotated to allow the rat to be inserted from above, and is then lowered and held in place by a set screw with a knurled head.

The Animals

The animals were Wistar white rats and were all fully mature males as these had been found to tolerate the highest dose rate—10 mg/kg—better than females or younger males.

The Radioiodine

In the first three experiments a dose of 25 μc was given intraperitoneally to each rat. In the fourth experiment this was reduced to 15 μc and in the last experiment to 8 μc. The dose was measured by means of the polythene phantom shown in Fig. 2, which consists of a test-tube holding the radioiodine in 0.1 ml of water immersed in the outer vessel to a distance of 4 mm from the bottom. With this arrangement a count of about 1/sec/μc was measured with the G.M. Counter and about 33/sec/μc with the scintillation counter.

The Measurement of Radioactivity

The early experiments were made with the G.M. Counter and Castle described elsewhere for use with sheep (Wright, 1958) and the later with an Ekco N 599A Scintillation Counter with the iron collimator replaced by a lead one 2 in. thick. The distance from the crystal to the animals' neck was 19.6 cm. All counts were made over a period of 100 sec.

The Chlorpromazine

Chlorpromazine hydrochloride (Largactil) in 0.5 or 1.0% solution was used at three different levels, 2.5, 5, and 10 mg/kg body weight.

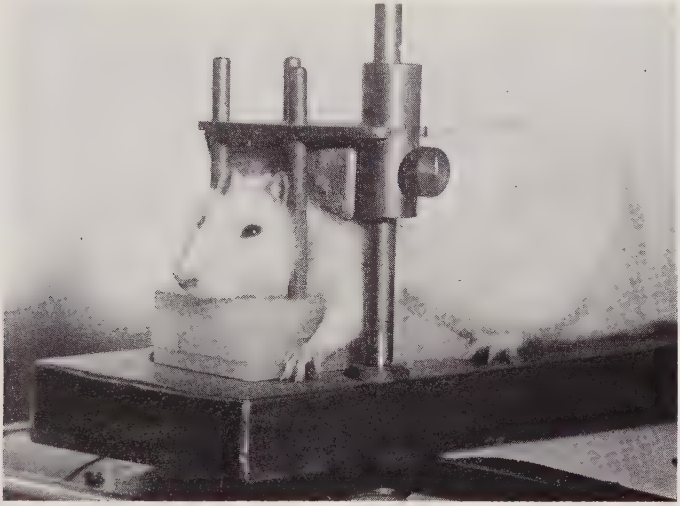


Fig. 1.—The rat holder.

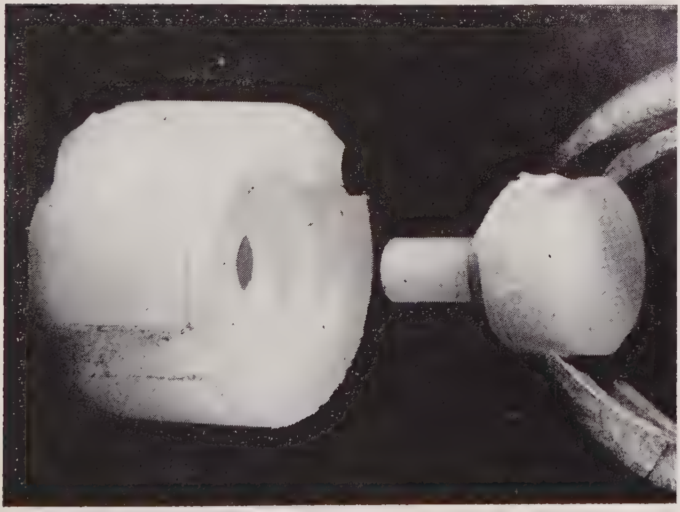


Fig. 2.—The phantom rat thyroid.

Except in the daily-dosed group of the last experiment, the drug was given intraperitoneally one hour before the radioiodine, as preliminary trials had shown that one hour was required for maximum effect. In the last experiment one group was given 2.5 mg/kg daily for 20 days, the last dose being given one hour before the radioiodine.

The Measurement of Body Temperature

In the fourth experiment, rectal temperatures were measured before each count up to 16 hours by means of a thermistor thermometer.

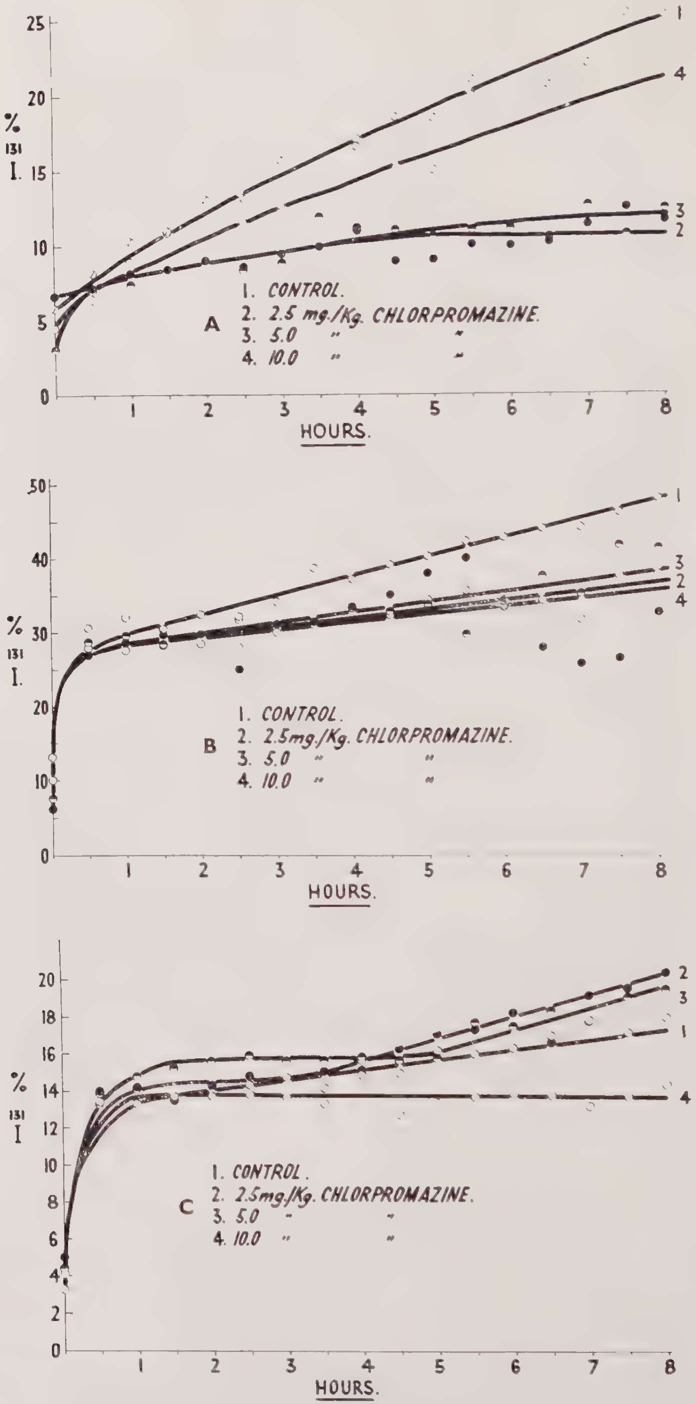


Fig. 3.—The effect of chlorpromazine on the accumulation of radioiodine by the thyroid of the rat. (First group of experiments.)

RESULTS

In the first three experiments four animals were used daily, one animal to each treatment, and this procedure was repeated for four days. In Fig. 3, A, B, C, the results are plotted, as a percentage of the dose of radioiodine absorbed, against time. Such differences as appear between treatments are not consistent in the three experiments and an analysis of variance of the combined results does not show any significant difference at any time. It was, therefore, concluded that, under these conditions, chlorpromazine at a dose rate of 2.5 mg/kg was without effect on the accumulation of radioiodine in the thyroid. Later, however, when the introduction of a scintillation counter had improved the precision of the technique, and in view of the findings of Mayer *et al.* (1956), I made two further experiments which differed from the first group in several respects. In the first place, the increase in sensitivity resulted in a reduction in counting error from 4–5% to less than 1%. Secondly, each treatment group consisted of ten animals instead of four and all were treated together, and, finally, the measurements were carried on over a much longer period.

In the first of these experiments I compared three levels of chlorpromazine given as a single dose and the results are plotted in Fig. 4. The pharmacological effect of the drug is illustrated by the curves for mean rectal temperatures which were taken for the first 16 hours, but it is evident from inspection that there is no significant variation among treatments in their effect on the accumulation of radioiodine.

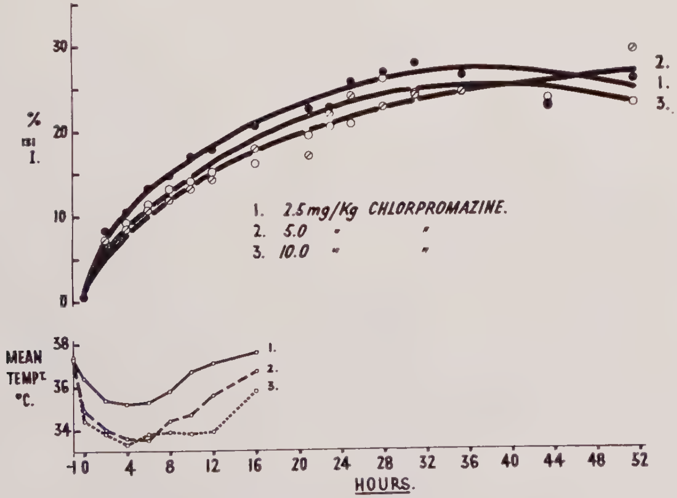


Fig. 4.—The comparison of single doses of chlorpromazine at various levels.

On the other hand, the results of the last experiment, which are shown in Fig. 5, indicate that, while a single dose of 2.5 mg/kg has no effect, daily administration of this amount of chlorpromazine causes a considerable inhibition of absorbtion of radioiodine. The apparent

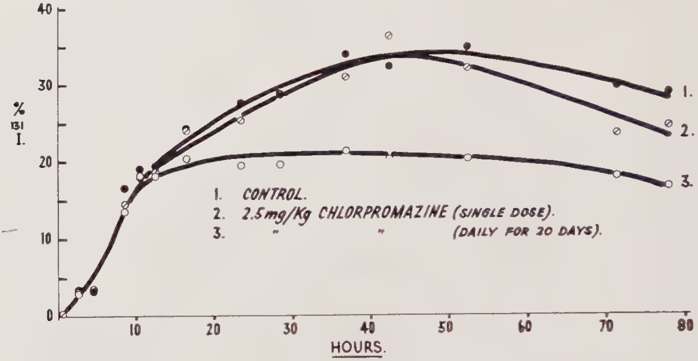


Fig. 5.—The effect of single and repeated doses of chlorpromazine.

divergence of the control and single-dose curves towards the end of the experiment is not statistically significant.

Discussion

The results should be considered in conjunction with those of Mayer *et al.* to which they are complementary. The latter found that when they added chlorpromazine to the incubation medium to a concentration of 0.036 mg/ml, it reduced the fixation of radioiodine by thyroid slices by about 20%. If the blood volume of the rat is taken as 7–8 ml/100 g a dose rate of 2.5 mg/kg should result in a blood level of about 0.03 mg/ml, in the absence of removal from the blood. As the visible signs of the drug wear off between 16–24 hours, the equilibrium level during prolonged daily dosing is probably also about 0.03 mg/ml. Therefore, my experiment would be expected to produce similar results to those of Mayer *et al.* which, in fact, it did, the inhibition being 30–40%. The absence of effect in the case of the single dose is probably due to failure of the drug to penetrate to the site of inhibition before biological decay has reduced the blood level to a negligible value, whereas, in the animal which has had time to reach equilibrium, the chlorpromazine will have reached a concentration at the active site equal to the plasma concentration which is sufficient to show the antithyroid effect.

In the curves of Fig. 5 the early rate of absorption and the rate of release are similar for all curves, but the daily-dosed group reached a maximum much earlier than the controls. This is indicative of the thiouracil type of goitrogen (Wright and Sinclair, 1958; and Wright, unpublished results).

Conclusions

The prolonged daily administration of 2.5 mg chlorpromazine per kilogramme body weight results in the partial inhibition of the absorption of radioiodine by the thyroid gland of the rat. This is in good agreement with the results obtained *in vitro* by Mayer *et al.*

When given as a single dose, at levels from 2.5 to 10 mg/kg and one hour before the injection of radioiodine, it is without effect and may be used for the control of rats in measurement of thyroid radioactivity.

ACKNOWLEDGEMENTS

The technical assistance of Mr. B. J. Roddick and the preparations of illustrations by Mr. A. L. Bryant are gratefully acnkowledged.

REFERENCES

COURVOISIER, S.; FOURNEL, J.; DUCROT, R.; KOLSKY, M.; KOETSCHET, P. 1953: Propriétés pharmacodynamiques du chlorhydrate de chloro-3 (diméthylamino–3′ propyl)–10 Phenothiazine (4.560 R.P.) *Arch. int. Pharmacodyn. 92:* 305-61.

DAS, N. N.; DASGUPTA, S. R.; WERNER, G. 1954: Changes of Behaviour and Electroencephalagram in Rhesus Monkeys Caused by Chlorpromazine. *Bull. Calcutta Sch. trop. Med. 1:* 5-6.

ESTRADA, E. 1956: Clinical Uses of Chlorpromazine in Veterinary Medicine. *J. Amer. vet. med. Ass. 128:* 292-4.

FISH, W. A.; CARLIN, T.; HICKEY, F. C. 1952: The Thyroidal Uptake, Retention and Iodine Pool in Rats under Controlled Iodide Diet. *Endocrinology 51:* 282-92.

KELLY, R. E.; LAWRENCE, D. R. 1956: Effect of Chlorpromazine on Convulsions of Experimental and Clinical Tetanus. *Lancet 270:* 118-121.

LOMAS, J. 1955: Uses of Chlorpromazine in Mental Hospital Patients. *Brit. med. J. (i)*: 879-82.

MAYER, S. W.; KELLY, F. H.; MORTON, M. E. 1956: The Direct Antithyroid Action of Reserpine, Chlorpromazine and Other Drugs. *J. Pharmacol. 117:* 197-201.

SMITH, A.; FAIRER, J. G. 1953: Hibernation Anaesthesia in Major Surgery. *Brit. med. J. (ii)*: 1247-8.

TROUGHTON, S. E.; GOULD, G. N.; ANDERSON, J. A. 1955: A Report on the Use of Chlorpromazine Hydrochloride in Domestic Animals. *Vet. Rec. 67:* 903-6.

VAN DEN HEEVER, L. W. 1955: The Use of Chlorpromazine Hydrochloride In an Equine Case of Tetanus. *J. S. Afr. vet. med. Ass. 26:* 301-3.

WASE, A. W.; GREENSPAN. J. 1953: Effect of 5-allyl-5 (1 methylbutyl) 2-thiobarbiturate on Uptake of ^{131}I by Rat Thyroid. *Proc. Soc. exp. Biol., N.Y. 84:* 154-5.

WEAVER, B. M. Q. 1956: A Report on the Use of Chlorpromazine Hydrochloride for Premedication in the Dog. *Vet. Rec. 68:* 347-9.

WRIGHT, E. 1958: The Concentration of Radioactive Iodine by the Thyroid Glands of Normal Sheep under Various Conditions. *N.Z. J. agric. Res. 1:* 224-30.

WRIGHT, E.; SINCLAIR, D. P. 1958: The Goitrogenic Effect of Thousand-headed Kale on Adult Sheep and Rabbits. *N.Z. J. agric. Res. 1:* 477-485.

A SURVEY OF COLLAR ROT AND ROOT ROT OF COX'S ORANGE PIPPIN AND OTHER APPLE VARIETIES IN NELSON AND MARLBOROUGH

By C. E. WOODHEAD, Pip Fruit Specialist, Department of Agriculture, Palmerston North

(Received for publication, 5 August 1957)

Summary

The results of a survey of collar rot and root rot in 69 Nelson and Marlborough apple orchards are presented.

Collar rot was recorded in 10.4% of single-worked Cox's Orange. Where topworked on other apple varieties, the incidence was only 2.6%. Collar rot was negligible in other varieties with the exception of Gravenstein, which had 2% infection.

Incidence of collar rot was correlated with age of trees, infection being highest in trees over 20 years. Poor drainage was the chief contributing factor.

Root rot was prevalent in trees over 40 years of age. Delicious, with 7.9%, was the most severely affected variety.

INTRODUCTION

Many Cox's Orange Pippin trees die each year in New Zealand apple orchards from the ravages of collar rot, caused by the fungus *Phytophthora cactorum.* Though diseased trees may be found in most if not all apple growing districts, loss of trees is especially heavy in Nelson Province.

In New Zealand the fungus responsible was first identified by Smith (1950). Attention is usually attracted to an infected tree in the first instance by failure of a portion of the tree to break into growth in spring. Alternatively, bud movement of one or more leaders may be late, subsequent growth is weak and the foliage sparse and yellow (Fig. 1). A superficial examination of the trunk may show nothing unusual but removal of the outer layer of bark just above the bud union will reveal an underlying area of rotted infected tissue, black or dark brown in colour, merging into lighter brown towards the margin of the canker where it joins healthy white tissue. A typical canker is illustrated in Fig. 2. Here infection has spread into the branches. As the disease progresses, the trunk is completely girdled and death of the tree follows. Although in Cox's Orange trees collar rot of the trunk *above* the bud union is the usual symptom of infection by *Phytophthora*, earlier observations on other varieties have indicated that this fungus attacks chiefly the roots and trunk below the bud union i.e. the portion of the tree provided by the rootstock.

The continuing heavy loss of Cox's Orange trees, coupled with the almost complete failure of remedial measures and the uncertainty of success when replanting in infected ground, are a serious threat to

N.Z. J. agric. Res. 1; 462–476.

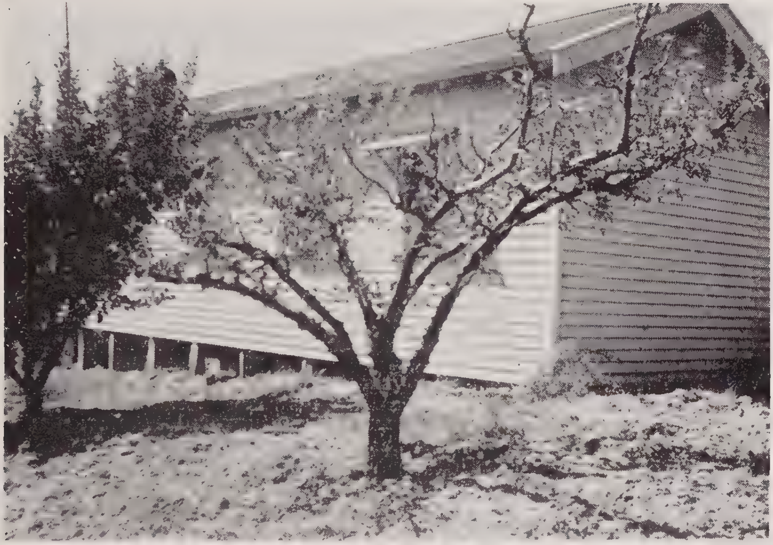


Fig. 1. Cox's Orange tree attacked by collar rot.

Photo: I. L. Brown

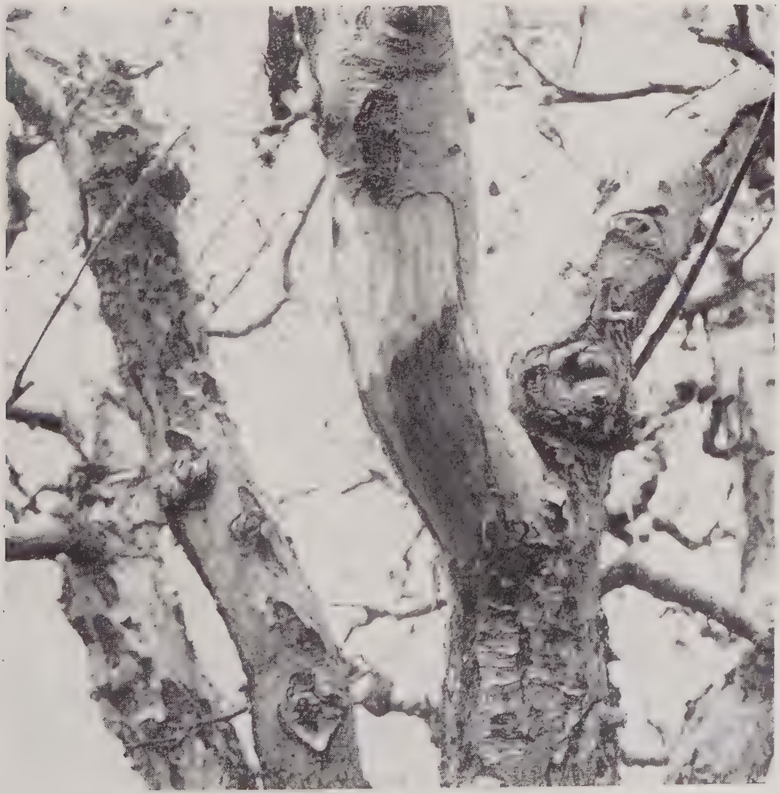


Fig. 2.—Cox's Orange branch infected with collar rot. Portion of bark removed to show canker.

Photo: I. L. Brown

production of this important export variety. To secure a more accurate estimate of the extent of collar rot in orchards and at the same time collect information that might indicate how the disease may be controlled or its incidence reduced, the survey described in this article was planned by the author and carried out by field officers of the Horticulture Division in Nelson and Marlborough during the spring and early summer of 1955–56.

SCOPE OF SURVEY

A survey of all Cox's Orange orchards being impracticable in the time available for the work, a sample selection was made of orchards at Nelson, Mapua and Motueka in Nelson Province and also at Blenheim in Marlborough. This selection was designed to give adequate representation of orchards on alluvial soils and on the Mapua clay typical of the Moutere Hills, on which most of the orchards in the three Nelson districts have been planted. As the former are flat or only slightly undulating and the latter gently to steeply rolling, this selection provided in addition for an estimate of the effect of ground slope on the incidence of collar rot.

Also included were a number of orchards in permanent grass, a recent development in orchard soil management. The number of orchards included and their distribution according to district, soil type and soil management are shown in Table 1. With reference to the grassed orchards listed, nine were actually included in the survey, but, as two orchards had been sown down only a few months previously they were classified as cultivated orchards.

TABLE 1. SCOPE OF SURVEY: DISTRICTS, NUMBER OF ORCHARDS, SOIL TYPES AND SOIL MANAGEMENT

Number of Orchards

	On Alluvial Soil			On Mapua Clay			
District	Cultivated	Grassed	Total	Cultivated	Grassed	Total	District Total
Mapua	8	1	9	11	—	11	20
Nelson	—	—	—	20	—	20	20
Motueka	6	3	9	8	3	11	20
Blenheim	9	—	9	—	—	—	9
Total	23	4	27	39	3	42	69

METHODS

When recording collar rot and root rot in the orchard, *Phytophthora* infection originating *above* the bud union was recorded as collar rot, infection *below* the union as *Phytophthora* root rot. Where root rot was the result of infection by another fungus or the cause could not be ascertained, infection was then recorded under the heading of "other root rots".

Identification of collar rot in the orchard presented few difficulties. In the time available it was, however, not practicable to examine the roots of all trees showing symptoms of root rot. The procedure followed here was to examine the roots of a sample number of infected trees in the orchard. Specimens of diseased roots from these trees were also forwarded to Plant Diseases Division for confirmation where possible of field diagnosis.

Many positive identifications of *Phytophthora* were made by Plant Diseases Division from these root specimens, indicating that this fungus was responsible for much of the root rot recorded. In many instances, however, the specimens forwarded were at an unsuitable stage for isolation of the fungus and in these cases the infection had to be classified as "other root rots". Because of these practical difficulties and also to give a clearer general picture of root rot as a whole in the orchards surveyed, *Phytophthora* root rot and "other root rots" are listed in the following tables under one inclusive heading.

In addition, when collar rot and root rot were recorded, trees were grouped according to age and whether the variety was single-worked or topworked. The term "single-worked" is used here to denote trees in which the variety had been budded or grafted directly on to the rootstock, in contrast to topworked trees where a previous variety had been reworked to the present variety. In the latter type of tree the trunk and usually portions of the main arms of the former variety remain to form an intermediate stem between the rootstock and the top variety. As will be shown later, this intermediate stem proved to be an important factor in the incidence of collar rot.

RESULTS OF SURVEY

Collar Rot Classification of Cox's Orange Orchards

In Table 2 Cox's Orange Pippin orchards included in the survey are grouped in various categories of collar-rot infection to show, firstly, the range of infection and, secondly, the seriousness of the disease in many orchards. This last point is well illustrated by the fact that in

TABLE 2: COLLAR ROT OF COX'S ORANGE PIPPIN: CLASSIFICATION OF ORCHARDS ACCORDING TO INFECTION*

Number of Orchards

Percentage of Infected Trees	0	Under 1	1–3	4–6	7–10	11 15	16 20	21 30	31–40	41–49	Total Orchards
Nelson	5	2	8	1	4	—	—	—	—	—	20
Mapua	—	—	1	2	4	1	3	4	1	4	20
Motueka	2	2	7	3	2	2	2	—	—	—	20
Blenheim	4	—	—	1	—	3	1	—	—	—	9
All districts	11	4	16	7	10	6	6	4	1	4	69

* Trees 5 years and under omitted. Classification based on infection recorded in both single-worked and top-worked trees.

15 of the 69 orchards more than 15% of the trees were infected, in four of them over 40%.

Incidence in Relation to Age and Type of Tree

Table 3 shows the extent of collar rot and root rot in single-worked and topworked Cox's Orange trees in various age groups. Considering first the figures given at the end of the table under the heading "All Districts" and with reference to single-worked trees, it will be noted that, of a total of approximately 18,600 trees, 10.4% were infected with collar rot. A significant feature is the relation of tree age to incidence, as shown by the progressive increase from 0.2% in the 0–5 age group to 17.7% in trees over 40 years. Turning to district figures for single-worked trees, Mapua with 22.6% has a much higher percentage of collar rot than other districts and more infection was recorded in Blenheim orchards than in those of Nelson and Motueka.

Attention is drawn to the low incidence of collar rot, 2.6% in the total for the four districts of 6,095 topworked trees. This is only a quarter of the infection recorded for single-worked trees and confirms earlier reports of field officers that Cox's Orange topworked on other varieties was relatively free from collar rot. Table 3 also indicates that, unlike single-worked trees, collar rot is negligible in topworked trees up to 40 years of age. Of 191 single-worked Cox trees on Spy stock in a Nelson orchard, 75 have been replaced owing to collar rot. Only one tree has been lost of the 58 Cox topworked on Statesman. Both blocks of trees were planted in alluvial soil and are growing under similar conditions.

Table 3 also shows that the incidence of root rot in single-worked Cox's Orange trees is very low by comparison with collar rot. In this connection a noteworthy feature of *Phytophthora* infection of the Cox's Orange trunk was that spread of infection downwards past the bud union, i.e. into the rootstock, was rarely observed.

Collar Rot in Other Varieties

It is clear from this survey that topworking Cox's Orange on other varieties gives a very large measure of protection against collar rot. However, if full advantage is to be taken of this knowledge in future planting, it is also necessary to know what varieties are likely to be satisfactory intermediates with respect to both resistance to collar rot and compatibility with Cox's Orange. Information recorded on these aspects during the survey is presented in Tables 4 and 5.

Dealing first with collar rot in single-worked trees of other varieties, Table 4 shows that infection was negligible in all varieties listed, with the exception of Gravenstein, which had 2% of trees infected.

With reference to Table 5, it should be explained here that intermediate varieties were graded for compatibility by observing the vigour and general health of the Cox's Orange topworked on them. It will be noted that the intermediate variety, Ribston Pippin, was more severely affected by collar rot than single-worked Cox's Orange. As Cox was raised from a seed of Ribston Pippin, its susceptibility to

TABLE 3. COLLAR ROT AND ROOT ROT OF COX'S ORANGE IN NELSON AND MARLBOROUGH ORCHARDS

Tree Age Group	Total Trees		Percentage with Collar Rot		Percentage with Root Rot	
	SW*	TW*	SW*	TW*	SW*	TW*
			Nelson			
0–5	665	—	0.2	—	0	—
6–10	—	—	—	—	—	—
11–20	—	—	—	—	—	—
21–30	423	58	1.2	0	0.5	0
31–40	3605	1003	4.9	0.1	0.3	2.2
over 40	34	67	2.9	0	8.8	8.9
Total	4727	1128	3.9	0.1	0.4	2.5
			Mapua			
0–5	713	186	0.3	0	1.9	0
6–10	29	—	10.3	—	0	—
11–20	30	—	0	—	0	—
21–30	15	—	33.3	—	0	—
31–40	—	—	—	—	—	—
over 40	4689	1713	26.1	7.1	0.1	1.0
Total	5476	1899	22.6	6.4	0.3	0.9
			Motueka			
0–5	748	318	0.3	0	0	0
6–10	104	71	1.0	0	0	0
11–20	284	—	2.8	—	0	—
21–30	328	—	7.3	—	0	—
31–40	2741	1488	5.8	1.8	0	0.2
over 40	2026	303	6.9	2.6	0	2.0
Total	6231	2180	5.8	1.6	0	0.4
			Blenheim			
0–5	121	315	0	0	0	0
6–10	23	—	0	—	39.1	—
11–20	—	246	—	0	—	16.3
21–30	—	—	—	—	—	—
31–40	—	—	—	—	—	—
over 40	2001	327	8.9	0	0.5	0
Total	2145	888	8.3	0	0.9	4.5
			All Districts			
0–5	2247	819	0.2	0	0.6	0
6–10	156	71	2.6	0	5.8	0
11–20	314	246	2.5	0	0	16.3
21–30	766	58	4.4	0	0.3	0
31–40	6346	2491	5.3	1.1	0.2	1.0
over 40	8750	2410	17.7	5.4	0.2	1.2
Total	18579	6095	10.4	2.6	0.3	1.5

* SW = Single-worked trees. TW = Top-worked trees.

TABLE 4. INCIDENCE OF COLLAR ROT IN OTHER APPLE VARIETIES
(Single-worked trees over 30 years*)

Variety	Total Trees	Infected Trees	Percentage of Total
Gravenstein	907	18	2.0
Delicious	17631	32	0.2
Jonathan	25578	12	0.05
Statesman	1835	1	0.05
Sturmer	26492	9	0.03
Dougherty	1034	0	0
Dunn's Favourite	1711	0	0
Cox's Orange Pippin (same age group)	15096	1880	12.4

* No infection was found in trees under this age.

TABLE 5. INCIDENCE OF COLLAR ROT IN COX'S ORANGE TOPWORKED ON DIFFERENT VARIETIES (Trees over 20 years of age)

Intermediate Variety	No. of Blocks	Compatibility No. of Blocks Good	Fair	Poor	Total Trees	Percentage Infected
Ribston Pippin	1	1	—	—	55	14.5
Sturmer	11	9	1	1	1535	5.1
Dunn's Favourite	11	8	3	—	1732	2.3
Statesman*	4	2	1	—	467	1.7
Premier	2	2	—	—	102	1.0
Scarlet Pearmain	1	1	—	—	120	0
Shorland Queen	1	1	—	—	117	0
King David	1	1	—	—	94	0
Worcester Pearmain	1	1	—	—	85	0
Emperor Alexander	1	—	—	—	40	0
London Pippin	1	1	—	—	30	0
Unknown	9	—	—	—	582	3.9
Cox's Orange Pippin single-worked, same age group	—	—	—	—	15862	12.1

* Compatibility of one block not recorded.

collar rot would appear to have been inherited from the Ribston parent. Table 5 also shows a fairly high incidence of 5.1% in trees on Sturmer intermediate, confirming the results obtained by Smith (1955) in inoculation tests on this variety. Nevertheless, the fact that Sturmer is much more resistant than Cox's Orange to collar rot is well illustrated in Fig. 3. Here healthy Golden Delicious topworked on Sturmer are seen in a Blenheim orchard growing in close proximity to a dying Golden Delicious topworked on Cox's Orange. Fig. 4 is a near view of the latter tree showing infection of the Cox's Orange intermediate by collar rot. Of the 66 Golden Delicious topworked on Cox in this orchard, 35 were infected with collar rot, in marked contrast to the

70 trees of the same variety topworked on Sturmer, all of which were healthy. This example clearly illustrates the risk involved in reworking Cox trees to another variety where collar rot is prevalent.

Although no infection was found in trees topworked on six of the intermediate varieties listed in Table 5, in each case only one block was available for observation. Consequently, less reliance can be placed on the data recorded for these varieties than for varieties where observations were made on several blocks and a larger number of trees. The only intermediate varieties in the latter category listed in Table 5 are Dunn's Favourite and Sturmer, each with 11 blocks of trees, and Statesman with 4 blocks. Trees on Dunn's Favourite and Statesman intermediates with respectively 2.3% and 1.7% infection are highly resistant when compared with single-worked Cox's Orange trees of the same age group, for which 12.1% infection was recorded. It would appear that either of these two varieties would provide a compatible and resistant intermediate in the future propagation of Cox's Orange trees.

Collar Rot and Soil Type

Collar rot recorded in Cox's Orange trees on alluvial and clay soils is shown in Table 6. The alluvial soils included several types of different origin with respect to parent material, the clay soils on the other hand were of one type only, the Mapua clay typical of the Moutere Hills. Infection figures for topworked trees have been omitted as their inclusion would tend to mask any effect of soil type.

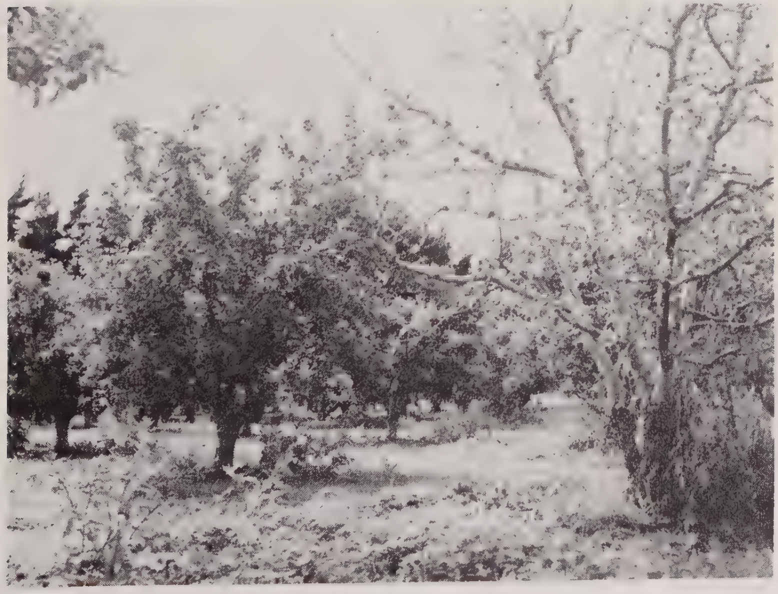


Fig. 3.—On left healthy Golden Delicious topworked on Sturmer. Tree at right Golden Delicious topworked on Cox's Orange dying from collar rot.

Photo: G. McCusker & Son, Blenheim

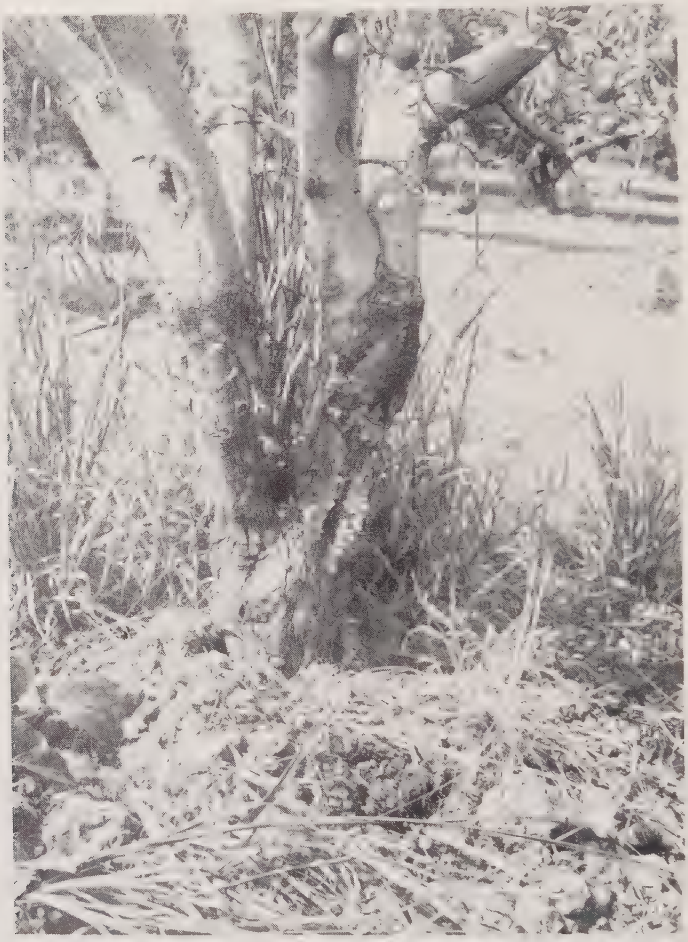


Fig. 4.—Near view of Golden Delicious on Cox in Fig. 3.
Photo: G. McCusker & Son, Blenheim

TABLE 6. COLLAR ROT INFECTION OF COX'S ORANGE PIPPIN ON ALLUVIAL AND CLAY SOILS (Single-worked trees only. Trees 5 years and under omitted)

District	Alluvial Soils*			Clay Soils†		
	Total Trees	Infected Trees	Percentage of Total	Total Trees	Infected Trees	Percentage of Total
Nelson	888	18	2.0	3174	165	5.2
Mapua	—	—	—	4763	1233	25.9
Motueka	1596	62	3.9	3887	269	6.9
Blenheim	2024	179	8.8	—	—	—
All districts	4508	259	5.7	11824	1667	14.1

* Several types.
† One type only, Mapua clay loam.

The results given in this table show that in the Nelson and Motueka districts collar rot was more prevalent on clay soils. It should, however, be noted that there was more collar rot on alluvial soils at Blenheim than in Nelson and Motueka orchards on clay. It is difficult to explain the very high incidence of collar rot on clay soil at Mapua in comparison with that recorded on the same soil at Nelson and Motueka, but the fact that the majority of trees at Mapua are over 40 years of age may be partly responsible.

Collar Rot in Grassed Orchards

Details of collar rot in Cox's Orange in the seven grassed orchards are given in Table 7. As only one of these orchards had been grassed for as long a period as three years and three were grassed in the year preceding the survey, no final conclusions can be drawn. Nevertheless, the amount of collar rot recorded in Orchards Nos 1–3 and 5 when considered in relation to history of infection before grassing, suggests that grassing has had no effect on the incidence of this disease. On the other hand collar rot apparently declined in Orchard No. 4 after grassing. In Orchard No. 6 the disease position is obscured by the treatment given to tree roots and trunks in the year of the survey. However, the weight of evidence appears to be against any effect of grassing.

TABLE 7. COLLAR ROT INFECTION OF COX'S ORANGE PIPPIN IN SEVEN GRASSED ORCHARDS (Single-worked trees over 5 years old)

Orchard No.	Year of Grassing	Percentage Trees Infected in 1955	Extent of Infection Prior to Grassing
1	1952	11.6	Similar
2	1953	4.8	Similar
3	1953	2.6	Not known
4	1953	0.9	Approximately 40% of trees replaced over period of a few years.
5	1954	2.6	No collar rot before grassing.
6	1954	0	Infection decreased progressively from 15% in 1950 to 4.6% at time of grassing. Earlier in the year of the survey tree trunks and main roots were treated with Bordeaux paste after removal of infected tissue.
7	1954	0	No collar rot before grassing

Other Factors of Environment and Management

It has been shown that tree age, topworking, and soil type influence the amount of collar rot found in orchards. In Table 8 soil type and various other features of environment and management recorded in the survey are listed for Cox's Orange orchards in two infection categories, i.e. up to 3% collar rot and from 11% to 49% collar rot. Reference to Table 2 reveals a considerable discrepancy between the two tables in the number of orchards listed in the "up to 3%" category. To explain this it is necessary to point out that the classification of orchards in Table 2 is based on infection recorded in one year only. It follows that where a policy of promptly removing and replacing diseased trees had been carried out, orchards with a recent history of severe infection could be, and some in fact were, included in a low infection category in the year of the survey. These orchards were omitted from Table 8. In addition, the omission of topworked trees when calculating infection percentages changed the classification of some orchards, causing a further reduction in the number of low-infection orchards. The group of 10 orchards shown in the first column of Table 8 therefore includes only those with a *past and present* history of no collar rot or a low incidence.

TABLE 8. ORCHARD AND MANAGEMENT FEATURES ASSOCIATED WITH SINGLE-WORKED COX'S ORANGE PIPPIN ORCHARDS IN LOW AND HIGH COLLAR-ROT-INFECTION GROUPS

	Orchards with up to 3% infection	Orchards with 11–49% infection
Number of orchards	10	21
On Mapua clay	5	15
On alluvial soil	5	6
On flat	6	9
On gentle slope	2	10
On steep slope	2	2
Poorly drained	1	12
Tree trunks buried in vegetation	6	12
Under irrigation	2	4
Pre-blossom copper sprays applied	5	8
Majority of trees with root-stock scion union at ground level or buried	7	17

The significance of factors listed in this table will be more readily seen if it is first noted that the number of orchards in the second group is approximately twice the number in the first group. Therefore, for a factor to be of any significance the figure in the second column must be appreciably greater or less than double the corresponding figure in the first column. Viewed in this way it is immediately clear that drainage is by far the most significant factor, as there is only one poorly-drained orchard in the low-infection group compared with 12 in the high-infection group.

Degree of ground slope also appears to be related to incidence of collar rot, as the number of orchards on gently sloping ground is proportionately very much higher in the high-infection group and the number on steep slopes proportionately less. It could be argued with reason that if slope, and its effect on drainage, is a significant factor one would expect to find a much higher proportion of orchards on flat land in this group. What the table does not show, however, is that field officers when noting special features associated with the particular location of collar-rot-infected trees, frequently recorded pockets of infected trees in wet areas at the foot of slopes, in hollows on slopes and in gullies between slopes. It will, therefore, be readily understood that wet "trouble spots" may occur with greater frequency in rolling country than on flat land.

As previously noted, soil type appears to be linked with collar rot incidence, the proportion of orchards on clay soil in the high-infection group being appreciably greater. As with slope of ground, however, the connection between soil type and drainage cannot be disregarded.

Other factors listed appear to be of little or no significance as figures in both groups are proportionately similar or differ only slightly. The general conclusion to be drawn from comparison of the two orchard groups is that poor drainage is the dominant factor in the incidence of collar rot in single-worked Cox's Orange trees. In support of this statement it may be mentioned that of the four single-worked Cox's Orange orchards that had a history of no collar rot infection—past or present—two were planted on a well-drained sandy loam and a third on dry, stony soil.

Under conditions of defective drainage, risk of infection must obviously be increased where the union of rootstock and Cox's Orange scion is at ground level or buried in the soil. This is clear from a comparison of collar rot infection in single-worked and topworked trees. In the course of the survey it was observed that, with a few exceptions, the initial infection by *Phytophthora* had occurred in the Cox wood at the bud union, which was in most instances at ground level or underground. In only a comparatively few trees was infection noted which had started at a point high up on the trunk or crotch, the maximum height recorded being 30 inches.

Root Rot in Other Varieties

The incidence of root rot in trees of the main varieties encountered in the survey is shown in Table 9. It will be observed that susceptibility to root rot increases with age of tree, little or no infection occurring in trees up to 10 years but increasing thereafter in a more or less regular pattern to the maximum recorded in trees over 40 years of age. The figures for this age group show Delicious as the most susceptible variety, closely followed by Gravenstein and Jonathan.

TABLE 9. INCIDENCE OF ROOT ROT IN DIFFERENT AGE GROUPS OF NINE APPLE VARIETIES (All districts: single-worked trees)

Age Group	Percentage of Trees Infected					
	0/5	6/10	11/20	21/30	31/40	Over 40
Delicious	0.2 (1389)*	1.7 (121)	5.0 (60)	1.0 (213)	2.8 (6764)	7.9 (10867)
Gravenstein	0.4 (892)	0 (10)	3.6 (195)	0 (50)	5.6 (161)	7.2 (746)
Jonathan	0 (1603)	0 (145)	0 (56)	0 (147)	2.6 (9095)	7.1 (16483)
Dunn's Favourite	—	—	—	—	0 (746)	6.2 (965)
Sturmer	0.04 (2748)	0 (134)	—	—	1.1 (10250)	5.4 (16242)
Golden Delicious	0.06 (1723)	0 (107)	2.0 (192)	4.9 (270)	—	—
Granny Smith	0.1 (1818)	0 (29)	0 (351)	3.4 (2040)	—	—
Dougherty	0 (97)	—	—	—	1.3 (302)	3.4 (732)
Statesman	0 (17)	—	—	—	1.0 (656)	3.3 (1179)

* Number of trees in age group.

In Table 10 details are given of root rot recorded in older trees of the three important varieties Delicious, Jonathan, and Sturmer in the four districts and on the two soil types. When districts are compared, the figures give a rather conflicting picture of the relation of disease to soil type and they are presented here mainly with the purpose of placing on record the amount of root rot noted in individual districts. Attention is drawn particularly to the high incidence in all three varieties at Mapua. The percentage of root rot recorded at Blenheim clearly shows that this disease is also serious in orchards on well-drained sandy loam.

The summary at the end of Table 10 indicates that root rot occurs in Delicious trees to a similar extent on both soil types but is more prevalent in Jonathan and Sturmer on clay soil.

The survey yielded little information on susceptibility to infection of different rootstocks, including East Malling types, although root rot was recorded separately for trees on different stocks. Trees over 10 years of age were almost invariably on Northern Spy stock and, as already indicated, root rot in trees under this age was too insignificant to attempt a correlation with rootstock type.

TABLE 10. DISTRICT INCIDENCE OF ROOT ROT IN OLDER TREES OF DELICIOUS, JONATHAN, AND STURMER, ON TWO SOIL TYPES

Variety	Percentage of Trees Infected					
	Delicious		Jonathan		Sturmer	
Age Group	31/40	Over 40	31/40	Over 40	31/40	Over 40
Nelson						
Alluvial Soils	3.0 (1745)*	11.0 (374)	7.0 (1990)	3.2 (725)	1.0 (2691)	0.6 (307)
Mapua clay	3.7 (2189)	—	1.8 (3930)	1.4 (216)	1.3 (4914)	6.0 (448)
Mapua						
Alluvial soils	—	—	—	—	—	—
Mapua clay	0 (51)	9.3 (6185)	—	11.8 (8076)	—	9.8 (7209)
Motueka						
Alluvial soils	1.2 (487)	1.9 (259)	0.5 (581)	1.4 (481)	0.4 (729)	1.2 (1214)
Mapua clay	2.3 (2292)	1.3 (977)	0.6 (2594)	0.3 (2067)	0.9 (1916)	0.9 (2673)
Blenheim						
Alluvial soils	—	7.5 (3072)	—	3.5 (4918)	—	2.4 (4391)
Mapua clay	—	—	—	—	—	—
All districts						
Alluvial soils	2.7 (2232)	7.5 (3705)	5.6 (2571)	3.2 (6124)	0.9 (3420)	2.1 (5912)
Mapua clay	2.9 (4532)	8.2 (7162)	1.3 (6524)	9.3 (10359)	1.2 (6830)	7.3 (10330)

* Number of trees in age group.

CONCLUSIONS

Collar Rot

The survey has shown that collar rot is a serious disease of single-worked Cox's Orange Pippin trees in Nelson and Marlborough orchards, incidence being especially high in the Mapua district. The disease is of little significance in other varieties with the exception of Gravenstein.

Though young trees are not immune, incidence of the disease in trees up to 20 years of age is low but under favourable conditions collar rot may become serious at any time after this age is reached.

It has been shown conclusively that topworking Cox's Orange on other varieties reduces the risk of infection by at least 75% and that collar rot is negligible in topworked trees up to 40 years of age.

Of the various factors of environment and management investigated, defective drainage appears to be dominant in increasing the disease. Though orchards showing high infection were most frequently located on clay soil and on gentle rather than steep slopes, it is probable that these factors are important only to the extent that drainage is detrimentally affected.

The evidence of grassed orchards, though inconclusive, does not suggest that grassing is an effective remedy.

There is also no clear indication that collar rot is increased where copper sprays i.e. pre-blossom Bordeaux, are not included in the spray programme.

Root Rot

The survey has also revealed a high incidence of root rot in trees over 40 years of the varieties Delicious, Jonathan and Sturmer, other varieties being affected in varying degree. It is also clear that the disease is prevalent in orchards on both light and heavy soils and must obviously play an important part in limiting the life of apple trees in commercial orchards.

ACKNOWLEDGEMENTS

Acknowledgement is due to all officers of Horticulture Division in Nelson and Marlborough who carried out the survey. Thanks are also due to Dr. F. J. Newhook, Plant Diseases Division, for identification of numerous disease specimens, to Mr. I. L. Brown, Horticultural Instructor, Nelson, for photographs used in Figs. 1 and 2, and to Mr. D. W. McCallum, Horticultural Instructor, Blenheim, for his assistance in connection with photographs used in Figs. 3 and 4.

REFERENCES

SMITH, H. C. 1950: Collar-rot of Apples and Gooseberries. *Orchardist N.Z., 23(11):* 11-14.

——————1955: Collar-rot and Crown-rot of Apple Trees. *Ibid. 28 (10):* 16-21.

THE GOITROGENIC EFFECT OF THOUSAND-HEADED KALE ON ADULT SHEEP AND RABBITS

By EVAN WRIGHT, Animal Research Station, Wallaceville, and D. P. SINCLAIR, Animal Research Station, Manutuke, Department of Agriculture.

(*Received for publication, 11 March 1958*)

Summary

Ewes that produced goitrous lambs as a result of grazing thousand-headed kale during pregnancy, also suffered interference with their thyroid functions. Adult rabbits were similarly affected by the kale.

Both spring- and autumn-sown plants were goitrogenic but rabbits showed less effect when fed on dried kale.

Radioiodine accumulated by the thyroids of the sheep grazed on kale, was discharged by thiocyanate, which indicates that the goitrogen of kale is of the thiouracil type.

INTRODUCTION

The occurrence of congenital goitre in lambs, without any clinical sign of thyroidal disorder in the ewe, when the latter has been grazed on thousand-headed kale, has been reported by Sinclair and Andrews (1954). In a previous paper (Wright 1958a) one of us suggested that an investigation with radioiodine might disclose some abnormality in the thyroid function of the ewe. In this paper we report the results of an experiment to relate this function with the occurrence of goitre in the lamb.

At the same time, in order to reduce the investigation, as far as possible, to a laboratory scale, we made an experiment to find out whether the rabbit was susceptible to the goitrogenic action of kale. Rabbits were chosen because they were most likely to accept kale as their sole food and because of the availability of a great deal of information on their thyroidal accumulation of iodine. (Brown-Grant 1954, 1955.)

If the goitre is due to a thyroid-blocking agent in the kale, the discharge of radioiodine from the affected gland by thiocyanate might provide a simple test for goitrogenic activity. It is well established that, if the ability of the thyroid to bind iodine as thyroglobulin is inhibited by a goitrogen such as thiouracil, inorganic iodide may still be concentrated by the gland, but may be discharged by thiocyanate (Vanderlaan and Vanderlaan 1947; Stanley and Astwood 1948; Berson and Yalow 1955). If this discharge were to be produced in goitrous animals midway between the times of administration and maximum thyroidal concentration of radioiodine, it should produce a curve of distinctive shape, whereas the control animals, not being subject to the inhibitor, would give normal curves. This would constitute a very useful test for the presence of a goitrogen with the advantage, firstly, that it would

N.Z. J. agric. Res. 1: 477–485.

show the presence of a certain type of goitrogen, the thiouracil type. Secondly, since the criterion would simply be the presence or absence of a particular and distinctive shape of curve, fewer animals would be needed than in the case where the criterion was the relative radioiodine concentration at a given time. We took the opportunity to test the feasibility of this procedure by adding a pilot experiment to the main experiment on sheep.

EXPERIMENTAL METHODS

The Kale

Two lots of thousand-headed kale (*Brassica oleracea* var. *acephala*) were grown at Manutuke. The first, referred to as "old kale", was planted in spring, grazed by fattening lambs in the summer and then shut up for use in this experiment in the winter. Though this crop was grazed by lambs it was already too tall for them to take the growing tops, so that it did not develop the thousand headed aspect. The second lot was sown in the autumn and its first grazing was by the experimental animals. This "new" kale was included since it is this kind of growth that causes congenital goitre in England (R. Allcroft pers. comm.). For the rabbit experiment, dried old kale was also used. This consisted of leaves stripped from the stalks and dried in a hot-air drier at 105°C.

The Sheep Experiment

Twenty-three Romney ewes were divided into groups and treated according to the plan shown in Table 1.

TABLE 1. SHEEP EXPERIMENTS: DETAILS OF TREATMENTS.

Group	Condition	Number of Sheep		Details of Grazing		
		Main Experiment	Pilot Experiment	Type of Kale	Days on Kale	Days on Pasture
A	Pregnant	5	2	Old	1–48, 55–56	49–55, 67–
B	Pregnant	4	1	New	1–48, 55–56	49–55, 67–
C	Pregnant	4	2	—	—	All
D	Empty	4	1	Old	1–66	67–

The break in the kale grazing period for groups A and B occurred during the period of lambing since this would be unmanageable on kale. The empty group, D, was included in the experiment in case this interruption reduced or masked the goitrogenic effect of the kale. The sheep were finally removed from kale on the 67th day, three days before injection of radioiodine.

Our knowledge of the physiological effect of different kinds of goitrogen was for a long time in a confused and contradictory state (Vanderlaan and Bissell 1946). As a result, one group of workers was even led to postulate a mechanism in which the roles of thiouracil and thiocyanate were reversed from those generally accepted today (Rawson *et al.* 1944). It is evident from the results of many people that the consequences of the intake of goitrogens, as measured by radioiodine accumulation, depend on the duration of treatment and more particularly on the time of measurement relative to the last dose of goitrogen. This has been more clearly shown recently (Butler *et al.* 1957). If the goitrogen thiocyanate is given up to and during the radioiodine measurements the maximum thyroidal concentrations of the radioiodine may be the same as that in untreated controls, whereas, if the goitrogen is withdrawn before the radioiodine is given, a much higher maximum results. On the other hand, given a short time before the radioiodine, a single dose of goitrogen will partly inhibit its absorption. Those results were obtained from small animals, but similar ones have followed the treatment of sheep with thiouracil (Wright, unpublished results). It was for this reason that the animals were removed from the kale before testing with radioiodine.

All lambs born during the experiment were killed at birth and their thyroids removed and weighed.

The measurement of the thyroidal accumulation of radioiodine was made by the methods described elsewhere (Wright 1958a). Twenty hours after the injection of radioiodine the six animals in the pilot experiment were dosed orally 6 g of potassium thiocyanate.

The Rabbit Experiment

Sixteen castrated male rabbits were housed in individual cages and fed *ad libitum* in four groups, each of which received one of the following diets: old kale, new kale, dried kale, or standard pellets as used in the rabbit colony. This part of the experiment, which lasted

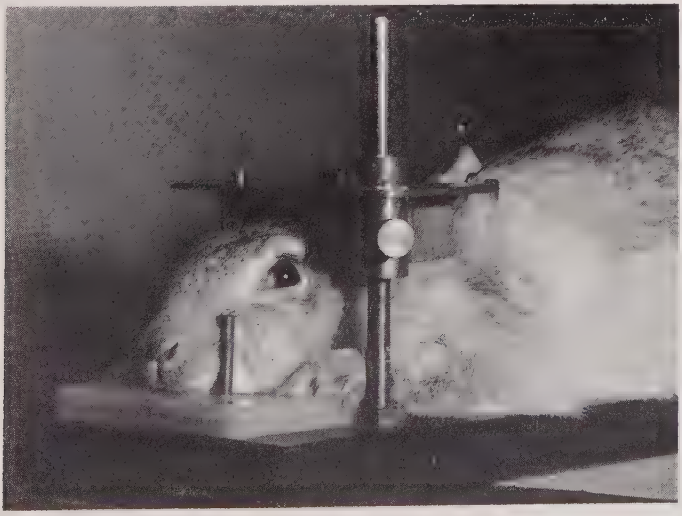


Fig. 1.—Measurement of thyroid radioiodine in the rabbit.

eight weeks, took place at Manutuke and then the rabbits were all placed on a diet of pellets and transferred to Wallaceville. Four days later, they were given an injection of radioiodine.

The apparatus for measuring radioactivity in the rabbit, which is shown in Fig. 1, is a modification of that used for rats (Wright 1958b). This arrangement does not immobilise the rabbit as does that of Brown-Grant (1954) but we have usually found in rabbits, and in other species also, that the easiest and most effective method of control consists of gentle handling combined with a minimum of physical restraint. Though the rabbit can escape if it struggles this is prevented by occasionally running the hand lightly down its back.

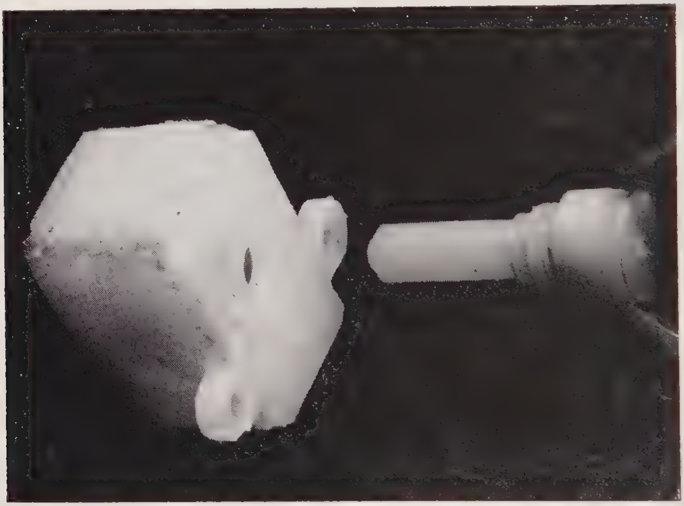


Fig. 2.—Phantom rabbit thyroid.

The dose of radioiodine is measured by means of the polyethylene phantom thyroid shown in Fig. 2. The radioiodine is placed in 0.5 ml of water in the test tube which is then immersed in water in the rectilinear vessel so that there is 0.5 cm of water below the test tube. The lugs on either side fit over the steel posts and ensure that the phantom is always in the same position. With this arrangement a dose of 25 μc gave a reading of 130 counts per second.

The radioiodine absorption measurements were carried out in lots of four, each lot containing one rabbit from each treatment group. They were given 25 μc of carrier-free radioiodine intraperitoneally in the morning and the raidoactivity in their thyroids was measured half hourly for twelve hours. Each measurement took five minutes.

Iodine Determination

The analyses of kale for iodine, for which we are indebted to Dr. G. W. Butler of the Plant Chemistry Division, Palmerston North, were made by the catalytic reduction of ceric ions by arsenious ions as described by Johnson and Butler (1957).

RESULTS AND DISCUSSION

The Sheep Experiment

The results of the main experiment are given in Fig. 3. If maximum curve height is taken as the criterion there is obviously no difference between pasture and old kale. The apparent difference between the new kale group and the two old kale groups is due to a low value for one of the four sheep in the new kale group and high value for one sheep in each of the old kale groups, and there is no significance in this difference when treated by statistical methods.

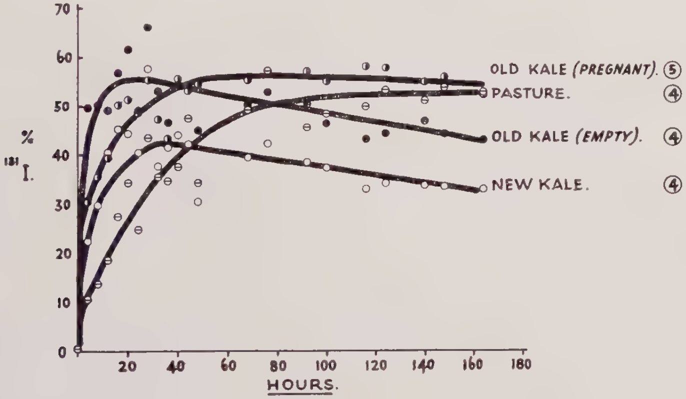


Fig. 3.—The effect of kale on the concentration of radioiodine in the sheep.

This is in contrast to the significant difference obtained between various treatments other than the intake of goitrogen (Wright 1958a). It is not to be concluded from this that the goitrogenic principle of kale cannot cause a difference in maximum radioiodine levels in the thyroids, but rather that under certain conditions this effect may be absent. Such a lack, therefore, is not necessarily an indication of the absence of a goitrogen.

On the other hand, the curves vary markedly in shape, both the rate of absorption and the rate of release being greater for the groups fed on kale. In these groups the maximum, though similar in magnitude to that of the controls, is reached very much earlier and a considerable amount of radioiodine had been discharged before the latter reached their peak. While the pattern for the pregnant ewes fed on kale is similar to the other treated groups as far as the time of reaching a maximum is concerned, it differs in showing a slower rate of release. The reason for this is not known.

This effect of increased rates of accumulation and release is indicative of the type of goitrogen which blocks the binding of iodine. This is well known in rats and one of us has obtained similar results from sheep treated with thiouracil.

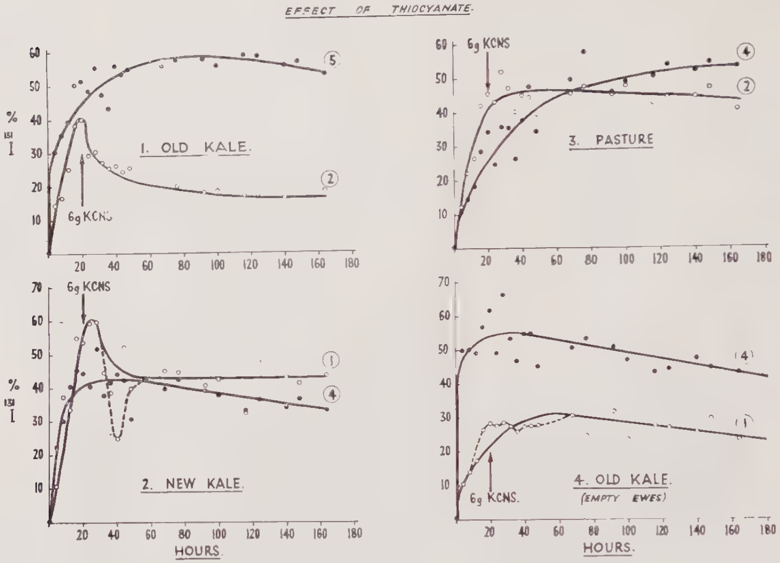


Fig. 4.—The discharge of radioiodine by thiocyanate in the sheep.

The conclusion that the goitrogen is of the thiouracil type is reinforced by the consequences of dosing with thiocyanate in the pilot experiment. The results of this experiment are shown in Fig. 4. The expected discharge of radioactivity is fully evident in the first graph. In the second group a similar, though lesser, effect is seen. If one neglects the high point at 40 hours the dotted curve fits the remaining points. Since we have had trouble at times from spurious counting caused by electrical interference and, as only one sheep is involved, this is probably justifiable. The control group showed no discharge while in the fourth group there may be some small effect shown by the dotted curve. This result is of little value as the one sheep involved lost weight during the experiment and died soon afterwards. It is probable that this sheep ate little or no kale and hence did not receive very much goitrogen.

TABLE 2. Iodine Content of "Old" Kale.

Date of Sampling	Iodine Content Expressed as μg/100 g Dry Weight
19 Dec., 55	120
25 Jan., 56	125
24 Feb., 56	120
13 Apr., 56	119
9 Jul., 56	60
14 Jul., 56	70

That the goitrogenic activity of the kale is not associated with a deficiency of dietary iodine is evident from the figures for the iodine content of the "old" kale given in Table 2.

The iodine content of the kale though much lower at the time of the experiment than it had been in the summer was still in the higher part of the range given by Johnson and Butler (1957) for various pasture species and since species with much lower values do not produce goitre it may safely be assumed that, unless its iodine is very much less available than that of other species, kale supplies this element in amounts which, in the absence of a goitrogen, should be adequate for the grazing animal.

The weights of the lambs' thyroids, listed in Table 3, show a clear difference between groups. The bracketed figure 5.5 g in the control group was obtained from a lamb found dead with only its head born, the head and neck including the thyroid being grossly distended. It was one of twins, the other lamb having a thyroid of 0.7 g. This abnormal figure is not included in the mean.

TABLE 3. WEIGHTS OF LAMBS' THYROIDS.

Group	Individual Weights in Grammes	Mean Weight
Old kale	3.5, 2.4, 12.2, 2.1, 3.5, 2.0	5.1
New kale	2.7, 4.4, 10.6, 9.4, 11.9, 8.4, 9.4	8.1
Pasture	(5.5), 0.7, 1.0, 1.6, 1.1, 1.0, 0.9, 1.2	1.1

The Rabbit Experiment

In the rabibt experiment, measurements of radioiodine accumulation were made on the rising part of the curve only. In Fig. 5 these are plotted in such a way that they can be compared directly with the curves obtained from sheep.

In this case the kale had a profound effect, increasing the absorption at 12 hours from 20% to 60% in the case of old kale and 70% in the case of new. The dried kale had much less effect, but the obvious reason for this, loss of goitrogen during drying, may not be the correct one as this food did not appear to be very palatable and a much smaller quantity of dry matter was eaten by this group than by those who received fresh material.

Though measurements on the rabbits were not made after twelve hours, the curves we obtained up to this time are similar in shape to those from the sheep experiment. The maximum heights are very different, but this difference might have been much less or even absent had we carried the measurements on longer. If the greater effect observed in the rabbits is real, it is probably due to the fact that they were exposed to the goitrogen longer, physiologically speaking, than were the sheep.

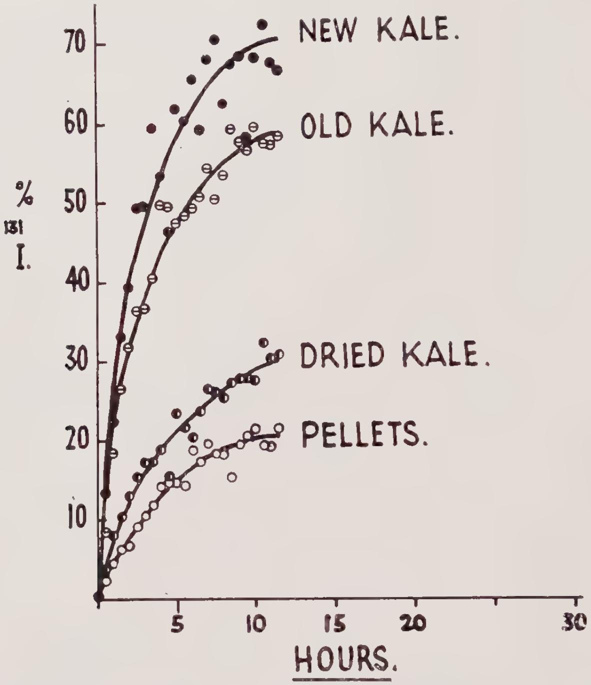


Fig. 5.—The effect of kale on the concentration of radioiodine in the rabbit.

Conclusions

It is apparent that, though the mothers of goitrous lambs show no clinical goitre, they still suffer considerable interference with thyroid function. Rabbits, too, are susceptible to the goitrogen of thousand-headed kale.

In both cases kale planted either in spring or autumn is effective in changing the thyroid metabolism, but when the kale is dried before being given to rabbits its effectiveness is reduced either because of loss of goitrogen on drying or because of reduced intake on account of unpalatability.

Since the radioiodine was discharged from the thyroid glands of the sheep by thiocyanate, the goitrogenic substance of the kale must belong to the thiouracil group, that is it inhibits the organic binding of iodine rather than the absorption of inorganic iodide.

As an indicator of the presence of the goitrogen two phenomena may be considered; firstly, the changes of gradient of the curve for thyroid activity and, secondly, the discharge of accumulated radioiodine brought about by potassium thiocyanate. Of these two criteria the latter has the advantage of appearing as a complex change of shape of the curve, rather than as a change in gradient. This change in kind rather than in degree should be measurable in single animals.

ACKNOWLEDGEMENTS

We are grateful to Mr. B. J. Roddick for technical assistance and to Mr. A. L. Bryant for preparing the illustrations.

REFERENCES

BERSON, S. A.; YALOW, R. S. 1955: The Iodine Trapping and Binding Function of the Thyroid. *J. clin. Invest. 34:* 186-204.

BROWN-GRANT, K.; GIBSON, J. G. 1955: Factors affecting the Uptake of Radioiodine by the Rabbit Thyroid. *J. Endocrin. 12:* ii.

BROWN-GRANT, K.; VON EULER, C.; HARRIS, G. W.; REICHLIN, S. 1954: The Measurement and Experimental Modification of Thyroid Activity in the Rabbit. *J. Physiol. 126:* 1-28.

BUTLER, G. W.; FLUX, D. S.; PETERSON, G. B.; WRIGHT, E. W.; GLENDAY, A. C.; JOHNSON, J. M. 1957: Giotrogenic Effect of White Clover (*Trifolium repens* L.) II. *N.Z. J. Sci. Tech. A38:* 793-802.

JOHNSON, J. M.; BUTLER, G. W. 1957: Iodine Content of Pasture Plants. 1. Method of Determination and Preliminary Investigation of Species and Strain Differences. *Physiol. Plant. 10:* 100-11.

RAWSON, R. W.; TANNHEIMER, J. F.; PEACOCK, W. 1944: The Uptake of Radioactive Iodine by the Thyroids of Rats made Goitrous by Potassium Thiocyanate and by Thiouracil. *Endocrinology 34:* 245-53.

SINCLAIR, D. P.; ANDREWS, E. D. 1954: Goitre in New-born Lambs. *N.Z. vet. J. 2:* 72-9.

STANLEY, M. M.; ASTWOOD, E. B. 1948: The Accumulation of Radioactive Iodide by the Thyroid Gland in Normal and Thyrotoxic Subjects and the Effect of Thiocyanate on its Discharge. *Endocrinology 42:* 107-23.

VANDERLAAN, W. P.; BISSELL, A. 1946: Effects of Propylthiouracil and of Potassium Thiocyanate on the Uptake of Iodine by the Thyroid Gland of the Rat. *Ibid. 39:* 157-60.

VANDERLAAN, J. E.; VANDERLAAN, W. P. 1947: The Iodide Concentrating Mechanism of the Rat Thyroid and its Inhibition by Thiocyanate. *Ibid. 40:* 403-16.

WOLFF, J.; CHAIKOFF, I. L.; TAUROG, A.; RUBIN, L. 1946: The Disturbance of Iodine Metabolism Produced by Thiocyanate. The Mechanism of Its Goitrogenic Action with Radioactive Iodine as Indicator. *Ibid 39:* 140-5.

WRIGHT, E. 1958a: The Concentration of Radioactive Iodine by the Thyroid Glands of Normal Sheep under Various Conditions. *N.Z. J. agric. Res. 1:* 224-30.

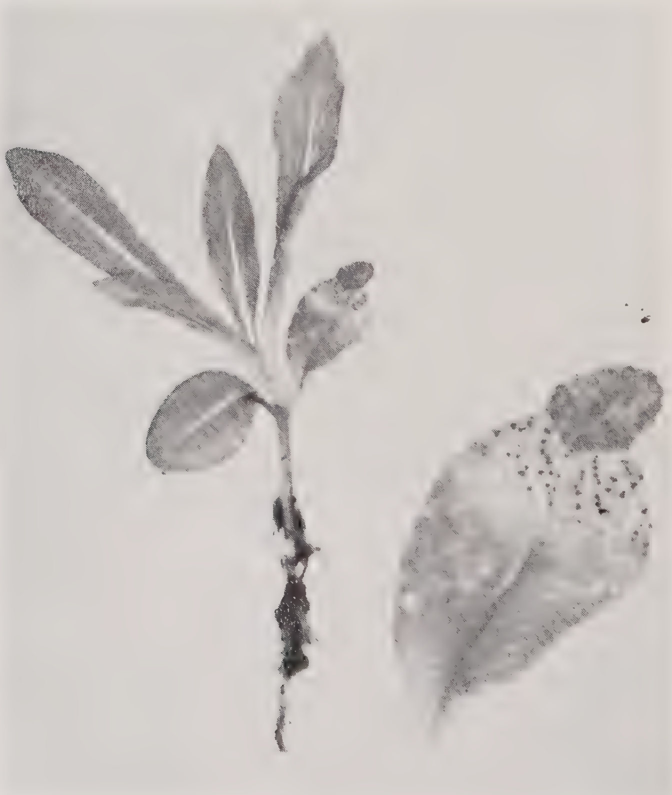
———— 1958b: The use of Chlorpromazine in the Measurement of Radioactive Iodine in the Thyroid Gland of the Rat. *Ibid. 1:* 455-61.

Septoria drummondii Ell. et Ev., A COMMON SEED-BORNE PATHOGEN IN EUROPEAN LINES OF *Phlox drummondii* Hook. SEED IMPORTED TO NEW ZEALAND

By H. T. Wenham, Massey Agricultural College, Palmerston North

(*Received for Publication, 10 March 1958*)

Septoria leaf-spot disease caused by *Septoria drummondii* Ell. et Ev. has for several seasons severely limited production of *Phlox drummondii* seedlings in commercial nurseries of the Manawatu. The disease is most severe during periods of prolonged cool humid weather. First symptoms are apparent approximately thirty-five days from sowing as diffuse chlorotic blotches on one or both cotyledons. Within three days these develop as distinct light-brown lesions covered with pycnidia (Fig. 1). On true leaves, first lesions may occur within fifteen days of first appearance of pycnidia on cotyledons. All seedlings from a seed

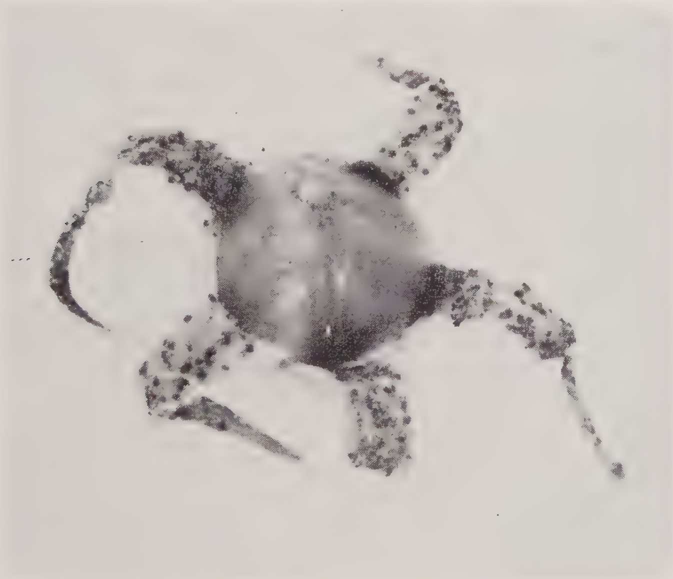


—*Photo: H. Drake.*

Fig. 1.—Infected seedling showing cotyledonary lesion and pycnidia. Enlarged cotyledon on right.

N.Z. J. agric. Res. 1: 486–488.

box initially containing less than ten seedlings with cotyledonary lesions may be heavily infected within thirty days of pricking out. The disease has been located on established plants throughout summer and autumn but is then less spectacular. Leaves are mainly attacked but lesions with pycnidia have also been observed on stems, petioles, peduncles, and sepals (Fig. 2).



—*Photo: H. Drake.*

Fig. 2.—Mature ovary of *Phlox drummondii* with sepals bearing pycnidia of the causal fungus.

The frequent association of the disease with seedlings raised in sterilised soil suggested seed as the source of primary inoculum. To test this hypothesis seed of three European lines which had produced heavily diseased plants in local nurseries was sown in new boxes of sterilized soil and set out in a glasshouse which had not previously held *Phlox drummondii* plants. Within thirty-five days from sowing all boxes had produced some seedlings with lesions and pycnidia on one or both cotyledons. Following common nursery practice, all boxes were overhead watered. Within sixty days from sowing all plants in all boxes were severely diseased.

To determine the extent to which lines of *P. drummondii* seed imported from Europe carried infection, forty-two lines were screened over three years by the following methods:

1. A 3-g sample of each seed line was vigorously shaken in 15 ml of sterile water and the washings sprayed over four healthy potted plants which were then held for fifty-six hours in a humidity cabinet before returning to the glasshouse. As controls, four potted healthy plants were atomised with sterile water. Within fifteen days, symptoms

had developed on plants of four series, whereas all "control" plants remained disease free.

2. Each seed sample was washed for a further ten hours in free running water to remove any loose surface inoculum, dusted with Fernasan A and then sown in new boxes of sterilised soil. By the fortieth day from sowing some seedlings with cotyledonary lesions and pycnidia were present in twenty-one boxes.

These results indicate that 50% of the lines tested carried viable inoculum of the causal fungus. Further, they show that inoculum may be present either in loose form contaminating seed, or be deep seated within the seed, the latter method being more frequent.

Although septoria leaf spot of *Phlox drummondii* caused by *Septoria drummondii* Ell et Ev. is world-wide in distribution it has not previously been recorded as a seed-borne disease.

NON-TOXICITY TO ANIMALS OF RYEGRASS ENDOPHYTE AND OTHER ENDOPHYTIC FUNGI OF NEW ZEALAND GRASSES

By I. J. Cunningham, Animal Research Station, Department of Agriculture, Wallaceville.

(*Received for publication, 11 March 1958*)

Summary

Feeding experiments have been carried out on birds and animals with perennial-ryegrass endophyte, tall-fescue endophyte, darnel endophyte, and the fungus that causes blind-seed disease of ryegrass. No toxic effects that could be ascribed to any of these fungi were produced in any of the experiments.

INTRODUCTION

Some of the fungi that grow on foodstuffs are poisonous to domestic stock, and familiar examples of such poisonous fungi are the ergots or sclerotia of *Claviceps purpurea* or *C. paspali.* The occurrence of toxic species has naturally enough led to the suspicion that other species may be similarly poisonous and, indeed, it has become the practice to assume that a fungus is toxic until the contrary is proved. When, therefore, Neill (1940, 1941) and Neill and Hyde (1939, 1942) discovered fungi associated with perennial ryegrass and other grasses in New Zealand, it became desirable to make tests of the effect of these fungi on animal health. This was specially important because of the widespread nature of the infection and the important part that one at least of the susceptible grasses, perennial ryegrass, plays in animal production in this country.

These new fungi have been fully described by Neill (1940, 1941) and Neill and Hyde (1939, 1942) and those examined for toxic effects are listed below with some descriptive comments extracted from Neill's articles.

Endophyte of Perennial Ryegrass

Vegetative forms of this fungus occur in leaves and seeds of perennial ryegrass and infection of a new generation of plants arises through sowing infected seed within 12 months of harvesting the seed.

Infection of ryegrass is widespread and no harm has been noted to the host plant.

Endophyte of Tall Fescue (Epichloe typhina (Pers.) Tul)

The vegetative forms affect the leaves and seeds of tall fescue. The infection is widespread and no adverse effect has been noted on the host plant.

Blind-seed Fungus (Gloeotinia (Phialea) temulenta (Prill. et Delacr.) Wilson, Noble, et Gray

This fungus occurs in the seed of perennial ryegrass and some of the fescues and causes marked reduction in germination. The infection appears to be confined to the seed head and has no influence on the plant apart from reduction in germination.

Endophyte of Darnel

This fungus is closely similar to those described above. Darnel has repeatedly been referred to in older literature as toxic to man and animals and trials with this fungus were carried out so that any positive findings could be used as a lead to the nature of the symptoms to be expected with the local fungi.

EXPERIMENTAL

Perennial Ryegrass Endophyte

Feeding experiments were carried out with cultures and with seed shown to be infected with endophyte.

Cultures

These were provided by Mr. J. C. Neill and were grown on rye and wheat grains inoculated on 23 May 1940. Moist infected grain was fed to sparrows and mice and dried ground infected grain was fed to mice. The moist infected grain contained about 30% dry matter.

Sparrow Feeding

Sparrows were trapped and kept in cages. Control animals were fed ordinary wheat grain and experimental animals were fed infected rye or wheat and given ordinary wheat grain when extra food was needed.

Controls. Five birds were kept from 16 August 1940. One bird died after 10 days, a second after 16 days and a third after 19 days. In each case death was preceded by about 24 hours of dullness and depression.

Experimental birds. (a) On 20 August 1940 two birds were fed 5 g of infected ryecorn taken direct from the culture tube. All was eaten within 4 hours and three hours later both sparrows were dull and feathers were ruffled. Both were dead next morning. There were no abnormalities noted at post-mortem examination.

(b) Two sparrows were fed 3 g infected ryecorn on 21 August 1940, 5 g infected ryecorn on 22 August 1940 and 10 g infected wheat grain on 23 August 1940. All was eaten quickly after feeding and supplementary normal wheat grain was fed on each day. The sparrows were well until the morning of 24 August 1940 when one was found dead though the second showed no symptoms and none appeared later.

Histological sections of liver, kidney, brain, and cerebellum from the dead sparrow showed no abnormality; lung sections showed some oedema of the cells.

Mice Feeding

Between 20 and 23 August 1940 three mice were fed 70 g of moist culture made up of 32 g of infected ryecorn and 38 g of infected wheat. All was eaten. There were signs of depression on the first day but these passed off and the mice were normal by the end of the feeding period.

Some remaining wheat culture was slowly air dried over a period of 2 months. This slow drying gave the maximal chance of toxin formation. In the course of drying, other moulds grew on the wheat. The air-dry material, which weighed 21 g, was ground and mixed with a solution of 2 g gelatine in 25 c.c. hot water and allowed to set to a cake. The cake was fed over 2 days to two of the mice which had previously received moist culture. No ill effects were observed.

These experiments showed that the cultures were not very toxic if indeed any toxin was present. The deaths in sparrows of the first experiment and the depression in the mice on the first day were more likely due to moist grain feed than to the presence of toxin.

Ryegrass Seed

The seed was a batch which showed 90% germination and 99.4% purity and was found by Mr. J. C. Neill to be about 100% infected with endophyte. High-germination seed was specially selected to avoid any interference from blind-seed fungus. The seed was ground and fed to mice, rats, and a sheep.

Mice and Rats

The diet employed was:

	Per cent
Ground ryegrass seed	80
Wheat germ	3
Dried blood	5
Dried yeast	1
Cod-liver oil	1
Mineral mixture	4
Gelatine	6

The diet was prepared by dissolving the gelatine in hot water and mixing this with the remainder of the ration. After cooling, the mixture set to a cake which could be fed with minimum waste.

Mice

Four mice were fed this ration from 21 October 1940 to 4 December 1940. Average weight of the mice was 28 g at commencement and 33 g at conclusion of the feeding period.

There was no evidence of toxicity except that in one mouse the lens of the right eye became opaque.

The animals were not slaughtered but were transferred to a diet of oats containing darnel and their further history is described in the section dealing with darnel.

Rats

Two groups of rats were fed the ration; these groups comprised 4 adult animals and 6 newly weaned animals.

Adult rats. The 4 rats were fed the ration from 21 October 1940 to 4 December 1940. Average weight was 198 g at commencement and 214 g at conclusion of feeding period. The rats were slaughtered and examined at the end of the experiment and no abnormalities were seen except that body fat was less than in stock rats of the same age. This was to be expected from the fibrous nature of the diet.

No histological examinations were made.

Young rats. The six young rats were fed from 31 October 1940 to 4 December 1940. Average weight at commencement of feeding period was 39 g and at conclusion was 103 g. Post-mortem examinations made on 4 December showed no abnormality except that body fat was less than in normally fed rats.

The growth rate was less than would be expected in rats on stock ration as such rats would have reached 130 g in the feeding period.

The smaller body weight could have been accounted for by the fibrous nature of the ration.

Sheep

An attempt was made to feed to a sheep ryegrass seed in a dry mixture of grain and chaff. The inclusion of the seed caused a drop in food consumption and to avoid loss of body weight from this cause, the ground seed was administered by dosing in suspension in water through a rumen tube and the sheep was run on pasture.

The doses given were 90 g per day from 22 November 1940 to 19 December 1940 and thereafter about 300 g on most days until 29 January 1941. The total dosed to the sheep was 10,800 g and no adverse effects were noted in the living animal. Body weight at commencement was 86 lb and 88 lb when the sheep was slaughtered on 6 February 1941.

No abnormalities were noted at post-mortem examination. Histological examinations were made of liver and kidneys. Kidneys were normal and liver sections showed some slight erosion of the lining of large bile duct but were otherwise normal.

The feeding experiments on sheep described under the section headed "blind-seed fungus" provided further evidence on the lack of toxicity of ryegrass infected with endophyte. Both the low-germination and the high-germination ryegrass seed used were about 100% infected with endophyte. No adverse effect was observed in one sheep which ate 12,900 g or in another which ate 9,400 g over a period of 74 days.

Tall-fescue Endophyte

Two adjacent areas of three-quarters of an acre were sown with tall-fescue seed in March 1943. The seed for one area was free from fescue endophyte and that used for the other was infected. A good germination and establishment of tall fescue grass was obtained. Examinations made in earlier years by Mr. J. C. Neill and later by Mr. R. M. Brien showed that the plants grown from endophyte-free seed were themselves free from endophyte while plants grown from infected seed remained infected until the termination of the experiment in 1945.

Cows were pastured on these experimental areas in three successive years.

In 1943 two cows were run on each area for 11 weeks from 2 April. For the last six weeks hay was fed and little food was obtained by the animals from the fescue.

In 1944 one cow was grazed on each area for 12½ weeks from 14 May onwards. The herbage was allowed to grow to about 1 foot high before the cows were put on and provided sufficient to maintain the animals with no supplementary feeding.

In 1945 two cows were grazed on each area for 7½ weeks from 15 May onwards. The herbage was grown to about 2 ft high before commencing grazing and there was sufficient to maintain the cows with no supplementary feeding. In that season there were considerable amounts of red clover and grasses other than tall fescue growing on each area.

The experiment was run mainly to determine whether the ingestion of fescue endophyte caused fescue lameness in cattle. General observations on health were made and detailed observations were made only on the condition of the legs. No lameness occurred nor was there any other adverse effect on health noted in any of the experimental cows.

Blind-seed Fungus

Feeding experiments were carried out on a sheep with ryegrass seed infected with the fungus which causes blind-seed disease.

A quantity of ryegrass seed of low germination (44%) was obtained from a seed merchant. For comparison seed of high germination (93%) was also obtained. The low-germination seed was examined by Mr. Neill and found to be heavily infected with the blind-seed fungus and the high germination seed was free from infection.

The ryegrass seed was fed to sheep mixed with chaff and crushed grain. Between 15 September 1939 and 27 November 1939 one sheep ate 12,900 g low-germination seed: a second sheep in the same period ate 9,400 g high-germination seed. Food intake was variable and on some days none was eaten—the weight of ryegrass seed consumed varied in consequence from nil on some days to 400 g on others.

No untoward symptoms were noted except that there was a small loss in weight resulting from food restriction enforced at some periods so as to ensure consumption of the seed.

The animal fed the low-germination seed was slaughtered at the end of the experiment. There were no abnormalities to be seen at post-mortem examination and the liver, kidney, and spleen were normal histologically.

Darnel

Feeding experiments were carried out on fowls, rats, sheep, and mice using as the source of darnel a quantity of imported hulled oats which contained 4.6% of darnel seed. The seed was infected with the darnel endophyte.

Fowls

Six birds of which 3 were in lay and 3 not laying, were fed the oats-darnel mixture from 13 November 1940 to 31 January 1941 in such a way as to include as much as possible in the ration.

Over this period the following feeding stuffs were consumed:

As mash: 75 lb oats, 24 lb bran, 12 lb meat-and-bone meal.

As grain: 50 lb oats.

The total consumption of oats was 125 lb and this contained 5.75 lb of darnel seed.

Food consumption was normal for age and size of birds. There was no evidence of lack of palatability of the ration or of any ill effect in the condition of the birds.

The three layers produced 93 eggs in the feeding period of 80 days which was similar to the production from normally fed stock birds of the same age and strain at that period of the year.

Rats

The oats containing darnel was incorporated in the following diet for feeding to rats and mice:

Oats (ground)	80
Gelatine	6
Dried blood	5
Wheat germ	3
Dried yeast	1
Cod-liver oil	1
Mineral mixture	4

The darnel content of this ration was 3.7%. The ration was made up by dissolving the gelatine in hot water and mixing this into the ration while hot. When the mixture cooled it formed a cake which could be fed with minimal loss.

Two groups of rats were fed:

(a) 5 half-grown males fed the ration from 21 October 1940 to 3 December 1940.

(b) 5 male and 5 female newly weaned rats fed the ration for the same period.

The average body weights in grammes at beginning and end of the feeding period were:

		At commencement	At end
Group (a)	5 males	197	290
Group (b)	5 males	41	188
	5 females	40	158

All animals were slaughtered on 3 December 1940. No abnormalities were noted at post-mortem examination. Four of the females in Group (b) were pregnant.

Sheep

One sheep was fed a mixture of oat straw, chaff and the oats containing darnel, from 15 November 1940 to 6 February 1941. In this period of 80 days the sheep ate 42 kg of oat straw chaff and 48 kg of oats which contained 2,200 g of darnel. Body weight of the sheep was 80 lb at commencement, 65 lb at the end of one month's feeding and 67 lb at the end of the experiment.

The animal was slaughtered at the end of the experiment and no abnormalities were observed at post-mortem examination or microscopically in the liver, kidneys, or spleen.

The loss of body weight was probably a reaction to the nature of the diet and the intake.

Mice

Five mice were fed the same ration as that used for rats, from 21 October 1940 to 5 December 1940. In this period the average body weight changed from 30 g at the commencement to 36 g at 5 December.

At that time the mice appeared well except that one mouse showed opacity of the cornea of both eyes. Feeding was therefore continued and 4 mice were added from an experimental group that had been fed ryegrass seed containing endophyte. A similar condition had developed in one mouse on the ryegrass seed ration and since no more ryegrass seed was available, it was decided to continue feeding with the ration containing darnel.

The new group of 9 mice were fed the experimental ration from 5 December 1940 to 30 April 1941. Average body weight was 35 g on 5 December and 38 g on 30 April. Examination of the eyes at 30 April 1941 showed that six animals were affected, two in both eyes. In 3 eyes there was opacity of the cornea, in two the lens was opaque and eyeball perforated and shrunken, in three eyes the eyeball was perforated and shrunken and the lens had disappeared.

The possible dependence of this eye condition on darnel feeding was examined by feeding mice a similar ration but with the darnel-containing oats replaced by normal hulled oats; 36 mice were fed this ration for 9 months. In six of these the eyeball became perforated and shrunken and the lens lost. There was no explanation of the cause of this eye condition which appeared to progress through the stages of opacity of the cornea, opacity of the lens, and perforation of the eyeball. The condition was not, however, specifically caused by feeding a ration containing darnel endophyte or ryegrass endophyte.

In a further experiment mice were fed a ration composed almost solely of darnel. Through the co-operation of Mr. E. Hullett of the Wheat Research Institute, darnel seed was ground and treated in every way the same as in making whole meal bread. This was done because of the suggestion that baking into bread in some way activates the toxin. The loaves of darnel were fed as the only food to a group of mice for a period of 3 weeks and no ill effects were produced in the mice.

DISCUSSION

The experimental work described showed that the fungi studied had no toxic effects. In the first feeding experiments there was some suggestion that cultures of ryegrass endophyte were poisonous to sparrows and to mice but this result was not confirmed in repeat experiments and could therefore be regarded as resulting from the rapid consumption of too large quantities of moist grain. Again the eye lesions produced in mice from feeding the ryegrass seed or darnel-contaminated oats were also produced by feeding the same ration but with pure oats replacing the darnel-containing oats. The eye lesions therefore were not a specific result of feeding fungus-infected food. These apparent toxic manifestations occurred in only a few of the experiments carried out and become of minor significance when compared with the greater number of experiments in which no toxic effects were produced.

Similar negative results were obtained by Neill (1942) from feeding different groups of chickens for short periods on ryegrass seed infected with ryegrass endophyte or with the fungus of blind-seed disease.

The possibility that the consumption of these fungi by grazing animals could be the cause of disease is therefore remote. The very wide occurrence of endophyte in ryegrass could not be overlooked as a possible contributing cause of facial eczema but this possibility has been eliminated by the absence of clinical signs or liver changes in sheep fed large quantities of infected seed and by the fact (J. C. Neill, pers. comm.) that facial eczema in the field has occurred on both infected and non-infected pastures. Again the experimental work has eliminated the tall-fescue endophyte as a possible cause of fescue lameness.

The negative results from feeding experiments with these fungi do, indeed, accord in a general way with experience with grazing stock as there is no consistent record of disease in circumstance under which the fungi are present in fodder.

Failure to produce toxic symptoms from feeding darnel was unexpected in view of the common belief—chiefly from ancient times, that darnel grain is poisonous (Pammel 1911; Long 1917).

The negative results obtained on the present work agree with observations made by Steyn (1933 a, b) in South Africa of absence of toxicity of darnel to horses, pigs, and rabbits. These negative findings are of interest to the present study because they confirm the results from feeding experiments with the similar or identical ryegrass endophyte.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Mr. J. C. Neill for providing cultures of ryegrass endophyte, for making many examinations of seeds and leaves for the presence of fungi and for reading the manuscript of this paper; to Mr. R. M. Brien for the examination of tall-fescue grass for endophyte, and to Dr. C. S. M. Hopkirk for histological examinations of animal tissue.

REFERENCES

LONG, HAROLD C. 1917: "Plants Poisonous to Livestock". C.U.P.

NEILL, J. C. 1940: The Endophyte of Ryegrass (*Lolium perene*) *N.Z. J. Sci. Tech. A21:* 280-91.

——————1941: The Endophytes of *Lolium* and *Festuca. Ibid. A23:* 185-93.

NEILL, J. C.; HYDE, E. O. C. 1939: Blind Seed Disease of Ryegrass. *Ibid. A20:* 281-301.

——————1942: Blind Seed Disease of Ryegrass—II. *Ibid. A24:* 65-71.

PAMMEL, L. H. 1911: "Manual of Poisonous Plants". Torch Press, Iowa.

STEYN, F. G. 1933a: Fungi in Relation to Health in Man and Animal. *Onderstepoort J. vet. Sci. 1:* 183-212.

——————1933b: Poisoning of Human Beings by Weeds Contained in Cereal (Bread Poisoning). *Ibid. 1:* 219-66.

TECHNIQUES OF MEASURING PASTURE PRODUCTION IN FERTILISER TRIALS

By I. L. Elliott, Rukuhia Soil Research Station, Hamilton, and P. B. Lynch, Department of Agriculture, Wellington.

(Received for publication, 26 February 1958)

Summary

A description is given of a series of experiments at the Rukuhia Soil Research Station where the responses of pasture to a number of fertilisers were measured by several different techniques. The techniques are compared as follows:—

1. As measures of total pasture production.
2. As measures of responses to fertiliser treatments.
3. Errors of estimation attached to each technique.
4. Effects of techniques on the species composition of the sward.
5. Effects of techniques on the chemical status of the soil.
6. Labour and land requirements of each technique.

For trials where stock production data are required in addition to pasture production data and where it is considered essential that treatments be compared under normal grazing management by stock, the "cage" technique as operated by the Extension Division is considered to be the most useful and reliable method. For small-plot trials where the primary aim is an estimation of fertiliser response by pastures, and where an accurate measure of total pasture production is not required, the "mowing and clipping returned" technique is satisfactory.

Certain limitations to the "mowing and clippings returned" technique are discussed.

INTRODUCTION

About £10,000,000 is spent annually by the farming community on fertiliser. Advice as to the best use of this money is frequently sought by individual farmers from the Instructors of the Extension Division of the Department of Agriculture. For the most part the advice given is based upon three things—experiments carried out locally by the Instructor, experiments carried out at centrally situated research stations, experimental areas, and demonstration farms, and the results of soil analyses related to past history and soil type.

It is quite obvious that the accuracy of the advice given to farmers will depend upon the soundness of the techniques employed in carrying out the trials upon which the advice is based. The essential purpose of the trials which will be described in this paper was to compare a number of techniques, all of which were employed in measuring the differences between treatments which were common to all trials.

In the early stages of experimental work on fertilisers and the determination of pasture responses to be obtained through their use on various soil types, there was little need for techniques capable of evaluating small differences. Simple observational trials, comparing two or perhaps three variables, were adequate at this time.

In the course of time it became important to evaluate differences between treatments more accurately and also to compare more variables within the one trial. For the most part this work fell outside the scope of the Instructor in Agriculture and became a major function of research areas and experimental farms.

When the Rukuhia Soil Research Station was first established in 1945, it was decided to make the investigation of techniques of pasture measurement a major activity. The techniques chosen for comparison were (1) "Cage" technique, (2) "Mowing with clippings returned" technique, (3) "White clover" technique, (4) "Alternate mowing and grazing" technique, (5) "Sears" technique.

In this paper the results of the work carried out between 1947 and 1956 are summarised.

DESCRIPTION OF TECHNIQUES EMPLOYED

Throughout the whole trial, comparisons between the techniques were made on treatments common to them all. These treatments were as follows:

(1) Control—no treatment.

(2) Superphosphate—4 cwt per acre annually.

(3) Superphosphate—4 cwt per acre annually plus lime 1 ton initially and 5 cwt per acre annually after the first application.

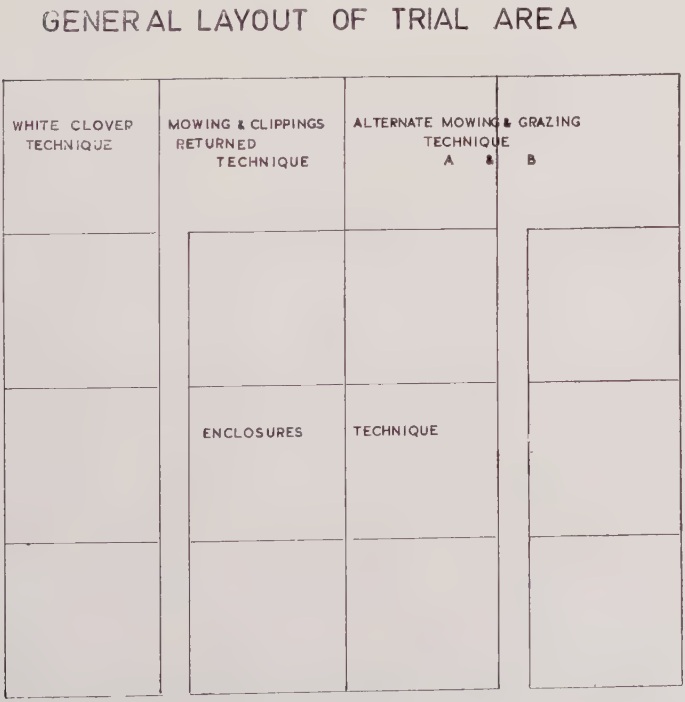

GENERAL LAYOUT OF TRIAL AREA
WHITE CLOVER TECHNIQUE
MOWING & CLIPPINGS RETURNED TECHNIQUE
ALTERNATE MOWING & GRAZING TECHNIQUE
A & B
ENCLOSURES
TECHNIQUE

Fig. 1.—General layout of trial area.

Since 1951, 1 cwt of Nitrolime per acre was applied annually to half the plots on the "mowing and clippings returned" and "Sears" sections of the trial.

The arrangement of the trial as a whole is shown in Fig. 1. During the second phase of the trial one section previously occupied by the "mowing and grazing" technique was used for the "Sears" technique.

The Enclosures or "Cage" Techinque (Lynch 1947)

In this technique each plot was separately fenced off and was grazed independently. The technique has two clearly demonstrable advantages over the others already referred to:

(1) The sward is constantly under control by the grazing animal and a sward most closely related to that developed under practical farming conditions is obtained.

(2) Transference of fertility through the animal is eliminated.

In connection with this latter point, special holding paddocks in which stock were depastured while not actually grazing the trial were given the same manurial treatment as the trial fields. Each treatment was grazed with a separate flock so that the animals grazing the superphosphate treatment, for example, were not at any time permitted to graze either the "super-lime" treatment or the "controls".

The size of each plot or enclosure was 1/10 acre. Production was measured by the use of a cage, 11 ft x 5 ft 6 in., which was moved at random round the enclosure.

One important point in connection with the operation of this technique is that after grazing the herbage should be allowed to recover above mowing height before the herbage protected by the frame is cut and a new area trimmed to receive the frame. In this way measurement of production is always from mower height to mower height.

"Mowing and Clippings Returned" Technique (Lynch 1947)

In this technique a mixed pasture was used and was laid out in 8 blocks of 3 treatments. In this case slightly larger plots were used than in the "white clover" technique and two strips each 24 in. x 25 ft were cut from each plot. Clippings were returned to the plots from which they were taken.

It should be pointed out that in all techniques which involve the return of clippings to mown plots, difficulty can be experienced from time to time in failure of the clippings to decompose between successive cuttings. This means that clippings may be harvested a second time and constitute a source of error. Such periods are, however, not usually of long duration, and if these circumstances arise, it is possible to overcome the difficulty by occasionally discarding the clippings.

"Mowing and Grazing" Technique

The method employed in this part of the trial was that first used by Hudson (1933) and subsequently described by Lynch (1947). The trial layout consisted of duplicate sections in which the treatments were arranged in a series of randomised blocks. Each plot was cut with a motor mower and the area harvested from each plot was 100 sq. ft. The cutting and grazing sequence used in this trial was similar to that outlined by Lynch.

"White Clover" Technique (Lynch 1947)

It had been observed in many previous experiments that when phosphatic or potassic fertilisers are applied to a mixed sward, the clover element in that sward is the first to be stimulated. This led to the view that clovers are more sensitive to applications of phosphates, potash, or lime, than are grasses, and, provided they could be kept as a pure sward, would provide a more sensitive measure of differences between such treatments than would be possible from a mixed sward of grasses and clover.

To refine the technique still further, the clover used in the trial was vegetatively reproduced from a single plant by cuttings. The original clover plant selected was one produced at the Grasslands Division of the Department of Scientific and Industrial Research and was known to be capable of yielding well. It was characterised by having a bright red coloration on the leaves and therefore it was an easy matter to eliminate from the plots plants not of this particular strain.

There were 8 blocks of three treatments and two strips 16 in. by 20 ft were cut from each plot for weighing. The clippings were returned.

The Proportional Return or "Sears" Technique (Sears 1944)

During the years 1951–53, comparisons were made between two areas, the plots upon which were treated identically. Production from one area was measured by the "mowing and clippings returned" technique, while production from the other was measured by the "Sears" technique. The "Sears" technique was conducted on one section of the area formerly used for the mowing and grazing trial.

The original treatments applied were maintained, but half of the plots received 1 cwt of the ammonium nitrate and lime mixture "Nitrolime" per acre annually. The following treatments were therefore available for comparison:

(1) Control.
(2) 1 cwt of nitrolime annually.
(3) 4 cwt of superphosphate.
(4) ,, ,, ,, + 1 cwt of nitrolime annually.
(5) ,, ,, ,, + lime.
(6) ,, ,, ,, + lime + 1 cwt nitrolime annually.

A complete description of the technique used was published by Sears (1944), but, briefly, the procedures involved are as follows:—

(1) The plots are mown and the weighed herbage is returned to the plots from which it was cut. The green herbage is sampled for dry-matter determination and botanical analysis.

(2) Following the mowing, sheep fitted with collecting bags and metal bottles are put on the plots in sufficient numbers to clean up the clippings and the unmown portion of the trial area down to mower height in 2 or 3 days.

The dung and urine collected from the sheep are measured and returned to the plots in proportion to the amount of dry matter yielded by each plot.

The main advantage of this technique is that while retaining the selective grazing effect of stock, it overcomes transference of fertility from plot to plot. However, it is time consuming, laborious, expensive to operate, and has limited applicability.

RESULTS

In the following tables, the techniques compared in the Rukuhia trials have been examined under the following headings:

1. As measures of total pasture production.

2. As measures of responses to fertiliser treatments (superphosphate and superphosphate plus lime in all cases and in addition the effect of the ammonium nitrate and lime mixture Nitrolime in the comparison of "Sears" and "mowings and clippings returned" technique.

3. Errors of estimation attached to each technique.

4. With the "cage" technique, comparisons of stock grazing and production data with herbage production.

5. Effects of techniques on species composition of the sward.

6. Effects of techniques on chemical status of soil as measured by "quick test" analyses.

7. Labour and land requirements of each of the techniques compared.

The trials can be considered in three groups:

First Period of 2½ years (1948-50) where comparisons are made between the "cage", "mowing and grazing", "mowing and clippings returned", and "white clover" techniques.

Second period (1951-53) where a comparison is made of "Sears" and "mowing and clippings returned" techniques.

Long-term comparison between "cage" and "mowing and clippings returned" techniques (1948-56).

Comparisons of Techniques as Measures of Total Pasture Production

One of the difficulties in making comparisons of this nature is that there is no "true" measure of pasture production. This figure is affected by the cutting technique employed, by the interval between cuttings, and by the general stock and pasture management (Lynch and Mountier 1954). In the trials examined in this paper, cutting is normally made at a comparable stage of growth, usually when 4 to 6 inches of growth are present, but the interval between cuts is not kept constant. Because of the need to cut trials under "mowing and clippings returned" technique before growth gets too long (when return of clippings becomes impracticable) it is probable that growth is cut under that technique, on the average, at a somewhat lower height than with the "cage" technique. This would have the effect of depressing production (Lynch and Mountier 1954) and may be one of the reasons for the lower pasture production figures shown by Table 1 for the "mowing and clippings returned" technique.

TABLE 1. HERBAGE D.M. (lb/ac) ON SUPER + LIME TREATMENT
FIRST PERIOD: 2½ YEARS

Year	Technique			
	Cage	Mowing and Grazing	Mowing and Clippings Returned	White Clover
1/4/48 to 20/5/49	14,750	12,470	11,490	6,370
21/5/49 to 18/4/50	14,840	9,440	9,470	7,250
1/6/50 to 30/11/50	6,010	4,910	4,750	4,220
Total 2½ years 1/4/48 to 30/11/50	35,610	26,810	25,610	17,860

Note: The apparent gap between 18/4/50 and 1/6/50 is explained by a change in the method of setting out the results. From 1/6/50 data were adjusted to exact 3-monthly seasonal periods, winter commencing from that date.

Table 1 shows that in this period the "mowing and clippings returned" technique has given about 72% of the production of the "cage" technique. The "mowing and grazing" method shows about 75% of the yields of the "cage" technique. The "white clover" technique yields about half that shown by the cage method. This is to be expected as the production of a white-clover sward is generally much less than that of a mixed pasture.

The "cage" method probably gives the closest approximation to production under rotational grazing with sheep, as cutting is made shortly after the sheep move out of the field and the pasture as a whole is not influenced by the cutting technique but is under "normal" sheep grazing. It would seem from Table 2 that the difference in production measurement over a long-term between the "cage" and the "mowing and clippings returned" technique is of the order of 20%.

In 1949-50, the production measured on "mowing and clippings returned" technique seems to have been relatively low (or that on the "cage" technique was relatively high). This has possibly overemphasised the differences between the "cage" and the other (small plot) techniques as shown in Table 1. Nevertheless it seems that the

TABLE 2. HERBAGE D.M. (lb/ac) ON SUPER + LIME TREATMENT LONG-TERM COMPARISON: "CAGE" AND "MOWING AND CLIPPINGS RETURNED"

Year	Cage	Mowing and Clippings Returned	
		Production	Relative to Cage = 100
1/4/48 to 20/5/49	14,750	11,490	79
21/5/49 to 18/4/50	14,840	9,470	64
1/6/50 to 31/5/51	10,580	Not available	
1/6/51 to 31/5/52	12,760	11,890	93
1/6/52 to 31/5/53	12,010	9,770	81
1/6/53 to 31/5/54	9,780	7,860	80
1/6/54 to 31/5/55	7,450	5,590	75
1/6/55 to 31/5/56	9,230	8,130	88
Total 7 years 1/4/48 to 31/5/56 (excluding 1950/51)	80,820	64,210	79

small-plot techniques compared here considerably under-estimate the total pasture production of a sward under rotational grazing by sheep.

Table 3 summarises results from trials comparing "Sears" and "mowing and clippings returned" techniques for the 2½ years (1951-53) when this comparison was made.

TABLE 3. HERBAGE D.M. (lb/ac) ON SUPER + LIME TREATMENT (Second Period: 1951-53) "SEARS" v. "MOWING AND CLIPPINGS RETURNED"

Year	Sears	Mowing and Clippings Returned
1/6/51 to 31/5/52	6,100	5,510
1/6/52 to 31/5/53	6,210	5,610
1/6/53 to 30/11/53	2,180	2,070
Total 2½ years 1/6/51 to 30/11/53	14,490	13,180

The figures in Table 3 do not represent total pasture production as with the "Sears" technique only every second cut could be weighed owing to the method of running the trial. The "mowing and clippings returned" figures are for those cuts which correspond to the cuts weighed on the "Sears" technique.

Over this period, therefore, the "Sears" technique has given yields about 10% greater than "mowing and clippings returned" method but, by inference, still somewhat below production figures as measured by the "cage" technique.

Comparisons of Techniques as Measures of Responses to Treatments

Tables 4 and 5 summarise the results that have been obtained with comparisons between the "cage" and small plot techniques while Table 6 compares responses measured on the "Sears" and "mowing and clippings returned" trials.

Note: In these tables:

M and G: refers to "mowing and grazing" technique.
M.C.: refers to "mowing and clippings returned" technique.
W.C.: refers to "white clover" technique.
Sears: refers to the proportional return technique developed by Sears.

TABLE 4. TREATMENT YIELDS OF DRY MATTER RELATIVE TO CONTROL = 100
(First Period: 2½ years)

Year	Response to Super				Response to Super + Lime			
	Cage	M & G	M.C.	W.C.	Cage	M & G	M.C.	W.C.
1/4/48 to 20/5/49	109**	108**	110**	109	116**	116**	120**	121**
21/5/49 to 18/4/50	109*	110**	112**	106	119**	113**	116**	107
1/6/50 to 30/11/50	118**	106**	110**	105	118**	109**	111**	104
Total 2½ years 1/4/48 to 30/11/50	111**	108**	111**	107*	117**	113**	116**	111**

* Differences significant at 5% level.
** Differences significant at 1% level.

Table 4 indicates that all techniques compared have measured significant responses to both superphosphate and superphosphate plus lime over control for the first $2\frac{1}{2}$ year trials, but with the "white clover" technique these effects are not significant for individual years except with superphosphate plus lime in 1948–49. In practically all other cases the difference is significant at the 1% level.

The "cage" and "mowing and clippings returned" techniques agree closely in estimating the size of responses. This is supported by the data in Table 5. The "mowing and grazing" technique in all cases estimates responses at slightly lower levels than the "mowing and clippings returned" technique.

Table 6 compares responses measured on "Sears" and "mowing and clippings returned" techniques for the period 1951-53.

TABLE 5. TREATMENT YIELDS OF DRY MATTER RELATIVE TO CONTROL = 100
LONG-TERM COMPARISON: "CAGE" AND "MOWING AND CLIPPINGS RETURNED"

Year	Response to Super		Response to Super + Lime	
	Cage	M.C.	Cage	M.C.
1/4/48 to 20/5/49	109**	110**	116**	120**
21/5/49 to 18/4/50	109*	112**	119**	116**
1/6/50 to 31/5/51	112*	Not Available	116**	Not Available
1/6/51 to 31/5/52	119**	114**	125**	117**
1/6/52 to 31/5/53	128**	120**	130**	128**
1/6/53 to 31/5/54	126**	115**	128**	123**
1/6/54 to 31/5/55	120**	114**	121**	116**
1/6/55 to 31/5/56	119**	130**	132**	145**
Total 7 years 1/4/48 to 31/5/56 (excluding 1950/51)	117**	118**	123**	125**

TABLE 6. TREATMENT YIELDS OF DRY MATTER RELATIVE TO CONTROL = 100
(Second Period 1951-53)
"SEARS" V. "MOWING AND CLIPPINGS RETURNED"

Year	Response to Super		Repsonse to Super + Lime	
	Sears	M.C.	Sears	M.C.
1/6/51 to 31/5/52	110*	115**	115**	119**
1/6/52 to 31/5/53	113**	113**	120**	119**
1/6/53 to 30/11/53	114**	106**	120**	114**
Total 2½ years 1/6/51 to 30/11/53	112**	113**	118**	118**

Nitrogen Responses. 1 cwt. Nitrolime per acre was applied to half the plots on 2/5/51, 16/5/52, and 25/5/53. As the response to this was short-lived, nitrogen responses are considered only for the winter period (June, July, August) following this application.

There is no evidence of differences between these two techniques as measures of superphosphate, superphosphate + lime, or nitrogen responses.

Winter	Mean Nitrogen Response	
	Sears	M.C.
1951	120	115**
1952	104	107
1953	112*	107
Mean 3 Winters	113*	110*

(Mean over "Control", P and P + Lime plots)

Errors of Estimation of Production Associated with Techniques

Tables 7, 8, ~~and~~ 9 show that all techniques compared have satisfactorily low errors of estimation associated with the measurement of yield. The "cage" technique has given the lowest errors and the "white clover" technique the highest with "mowing and clippings returned" technique in an intermediate position.

TABLE 7. COEFFICIENTS OF VARIATION ATTACHED TO PRODUCTION MEASUREMENTS

(First Period 2½ years)

Year	S.E. as Percentage of Mean Plot Yield			
	Cage	M. & G.	M.C.	W.C.
1/4/48 to 20/5/49	2.1	5.4	5.0	9.6
21/5/49 to 18/4/50	3.6	7.0	3.9	5.8
1/6/50 to 30/11/50	5.2	6.7	4.9	7.1
Total 2½ years 1/4/48 to 30/11/50	1.4	4.9	3.7	5.6

Livestock Data and Pasture Production from the "Cage" Technique

Table 10 gives the comparison of herbage yields and sheep grazing days on the fields from which samples were taken by the "cage" method for pasture production measurement.

During the 8-year trial period summarised in Table 10 there has been a decline in production and carrying capacity on the "control" treatment plots and an increase in the size of the responses measured to superphosphate and to superphosphate plus lime. The figures also suggest that there was a decline in dry matter consumed by sheep after the first two trial years. This may have been due to a different intensity of stocking in relation to the feed available or to the feed becoming progressively less palatable. However, the ratio for the superphosphate and superphosphate plus lime treatments is closely

TABLE 8. COEFFICIENTS OF VARIATION ATTACHED TO PRODUCTION MEASUREMENTS

LONG-TERM COMPARISON: "CAGE" AND "MOWING AND CLIPPINGS RETURNED"

Year	S.E. as Percentage of Mean Plot Yield	
	Cage	M.C.
1/4/48 to 20/5/49	2.1	5.0
21/5/49 to 18/4/50	3.6	3.9
1/6/50 to 31/5/51	5.4	Not available
1/6/51 to 31/5/52	6.2	5.1
1/6/52 to 31/5/53	4.0	4.7
1/6/53 to 31/5/54	4.3	5.5
1/6/54 to 31/5/55	6.6	5.5
1/6/55 to 31/5/56	4.5	4.7
Total 7 years 1/4/48 to 31/5/56 (excluding 1950/51)	1.4	3.9

TABLE 9. COEFFICIENTS OF VARIATION ATTACHED TO PRODUCTION MEASUREMENTS

"SEARS" v. "MOWINGS AND CLIPPINGS RETURNED"
(Based on comparable cuts)

Year	S.E. as Percentage of Mean Plot Yield	
	Sears	M.C.
1/6/51 to 31/5/52	6.5	6.7
1/6/52 to 31/5/53	3.6	4.3
1/6/53 to 30/11/53	7.7	4.6
Total 2½ years 1/6/51 to 30/11/53	4.8	4.7

similar to that of the control treatment which suggests that the intensity of stocking was the same on all treatments but changed over the years and that the palatability factor was not important.

As measures of treatment responses over yearly periods, sheep grazing data and herbage production measurements agree reasonably

TABLE 10. COMPARISON OF SHEEP GRAZING DAYS AND HERBAGE PRODUCTION

Year	Control Plots (per acre)			Grazing Days Relative to Control = 100		D.M. Production Rel. to Control = 100	
	Sheep Grazing Days	D.M. Production (lb)	Ratio D.M. to Grazing Days	Super	Super + Lime	Super	Super + Lime
1/4/48 to 20/5/49	3,770	12,700	3.4	113	121	109	116
21/5/49 to 18/4/50	3,860	12,520	3.2	108	106	109	119
1/6/50 to 31/5/51	3,400	9,120	2.7	123	125	112	116
1/6/51 to 31/5/52	4,330	10,200	2.4	126	127	118	125
1/6/52 to 31/5/53	3,790	9,250	2.4	124	124	128	130
1/6/53 to 31/5/54	2,930	7,660	2.6	126	123	126	128
1/6/54 to 31/5/55	2,520	6,160	2.4	126	130	120	121
1/6/55 to 31/5/56	2,900	6,990	2.4	130	132	119	132
Total 8 years 1/4/48 to 31/5/56	27,500	74,600	2.7	121	123	117	123

RATIO OF DRY-MATTER PRODUCTION TO SHEEP GRAZING DAYS

Treatment	1948/49	1949/50	1950/51	1951/52	1952/53	1953/54	1954/55	1955/56	Totals
Control	3.4	3.2	2.7	2.4	2.4	2.6	2.4	2.4	2.7
Super	3.2	2.9	2.4	2.2	2.5	2.6	1.9	2.2	2.5
Super + Lime	3.2	3.2	2.5	2.3	2.6	2.7	2.3	2.4	2.6

well. The main difference seems to be in the small and variable response to lime shown by the herbage yields and not always by the grazing figures. Fig. 2 compares the yearly responses summarised in Table 10. There is one anomalous reading (superphosphate + lime treatment 1949-50) but some indication that for responses in herbage dry-matter production above about 20% there is little corresponding increase in grazing-time measurements. This effect may be due to the

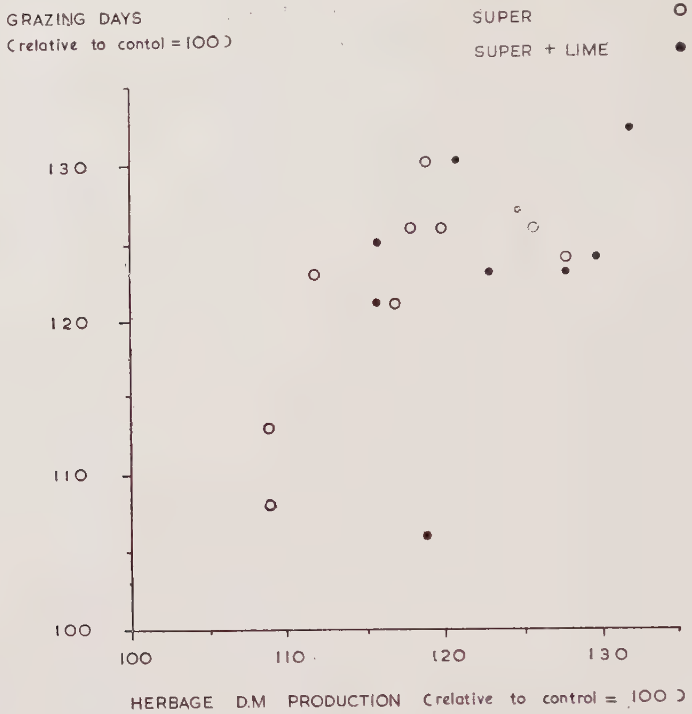


Fig. 2.—Yearly responses to treatments ("cage" technique) as measured by grazing days and herbage yields.

officers not taking advantage of yield increases above this level by increasing stock numbers. This could lead either to more wastage of feed on high-producing fields or to the increase being taken up in greater live-weight gains.

Unfortunately the data on live-weight gains of sheep are not complete but the available information is given in Table 11.

From Table 11 it would seem that the lime response has been quite marked at times in terms of live-weight gains per acre while the superphosphate response, particularly after 1952-53, is relatively small. The herbage dry matter required (as measured by the "cage" technique) per pound of live-weight gain seems to have varied for the most part between 8 and 15 lb, with a mean about 10 lb. The lower figure for the period August 1954 to January 1955 may be a reflection of lower maintenance requirements in spring and summer and consequently a greater efficiency in the conversion of grass to meat in this period. The trial at all times has been stocked with mature dry sheep of 100–150 lb live weight.

The information given in Tables 10 and 11 shows that the herbage production data from the cage technique are not seriously inconsistent with the livestock data as measures of treatment responses although there is no close correspondence and it is impossible to make critical

TABLE 11. Comparison of Sheep Live Weight Gains and Herbage Production
(Cage Technique)

Year	Sheep Weight Gains			D.M. Production Relative to Control = 100		Pounds Herbage Required (D.M.) for 1 lb L.W. Gain		
	lb/ac	Relative to Control = 100						
	Control	Super	Super + Lime	Super	Super + Lime	Control	Super	Super + Lime
10/4/48 to 10/6/49	1,080	118	120	107	116	8	8	8
13/5/52 to 6/5/53	720	113	165	132	136	13	15	10
6/5/53 to 30/6/54	1,040	105	116	125	125	8	10	9
10/8/54 to 24/1/55	580	107	135	115	122	6	7	6
6/5/55 to 9/6/56	800	97	112	120	128	10	12	11
Total all periods	4,220	108	127	120	126	9	10	9

comparisons with the available evidence. There is no measure of feed intake although it is planned to do this in the immediate future.

Effects of Techniques on the Species Composition of the Pastures

Tables 12, 13, and 14 examine changes that have occurred in the swards subjected to the various experimental techniques. The data are derived from species dissection analyses of the cut herbage after weighing for production measurements. The superphosphate-plus-lime and control treatments have been selected for comparison of techniques.

Not every production cut was analysed for pasture species composition and there are some gaps in the record particularly in the early years of the trials. In Table 12 (First Period) figures are available for the July-October 1950 period only. The figures in Tables 13 and 14 are for practically all cuts dissected on the "mowing and clippings returned" technique and for the comparable cuts on the "cage" method, A more complete series of analyses is available from the "cage" technique. The figures quoted are mean percentages of species based on dry weights. M. & G. refers to the "mowing and grazing" technique; M.C. to the "mowing and clippings returned" technique.

"Other grasses" are mostly low-producing volunteer species. Browntop (*Agrostis tenuis*) is common throughout the year and the amounts of the other volunteer grasses vary with the seasons; Yorkshire fog (*Holcus mollis*), *Poa trivialis* and *Poa annua* are more evident in the winter, and goose grass (*Bromus mollis*) is prominent in the summer. "Other species" are mostly weed species dominated by flat weeds such as *Plantago lanceolata* and *Leontodon hispidus* and in higher fertility areas by docks (mostly *Rumex obtusifolius*).

TABLE 12. MEAN PERCENTAGES OF SPECIES IN MOWN HERBAGE ON SUPER + LIME PLOTS—FIRST PERIOD

Species	Date Dissected (1950)	Technique		
		Cage	M. & G.	M.C.
Perennial ryegrass (*Lolium perenne*)	Jul.	72	—	30
	Aug.	75	85	—
	Sept.	70	78	54
	Oct.	73	—	69
Cocksfoot (*Dactylis glomerata*)	Jul.	6	—	26
	Aug.	4	2	—
	Sept.	2	2	12
	Oct.	2	—	4
Other grasses	Jul.	4	—	10
	Aug.	5	1	—
	Sept.	6	6	13
	Oct.	8	—	17
White clover (*Trifolium repens*)	Jul.	16	—	28
	Aug.	14	9	—
	Sept.	20	12	20
	Oct.	15	—	14
Other species	Jul.	2	—	6
	Aug.	2	4	—
	Sept.	2	2	2
	Oct.	2	—	Nil

Note: In this and subsequent tables (—) means "not available".

The "mowing and clippings returned" technique has less ryegrass and more cocksfoot and other grasses than the other techniques compared here. This is probably a chance effect resulting from original differences in sward composition on the trials. In the long-term comparison of the "cage" method with the "mowing and clippings returned" technique (Tables 13 and 14) this difference persists for the 6 years over which the comparison is made.

There seems to be no evidence of progressive sward deterioration in these two trials. However, an invasion of flat weeds (mostly *Plantago* species) on the "mowing and clippings returned" trial was successfully dealt with by spraying with the potassium salt of MCPA at 1 lb acid equivalent per acre on 9 April 1953. This invasion did not occur on the "cage" technique trial probably because these weeds are palatable to sheep. If one accepts treatment with weedkillers as a

TABLE 13. LONG-TERM COMPARISON: "CAGE" AND "MOWING AND CLIPPINGS RETURNED"
MEAN PERCENTAGES OF SPECIES IN MOWN HERBAGE ON SUPER + LIME PLOTS

Species	Date Dissected	Technique		Species	Date Dissected	Technique	
		Cage	M.C.			Cage	M.C.
Perennial ryegrass	Jul., 50	72	30	White clover	Jul., 50	16	28
	Oct., 50	72	69		Oct., 50	15	14
	Aug., 51	83	32		Aug., 51	12	23
	Oct., 51	78	—		Oct., 51	11	—
	Apr./May, 52	64	12		Apr./May, 52	26	50
	Nov., 52	70	24		Nov., 52	14	17
	Jan./Feb., 53	46	21		Jan./Feb., 53	31	56
	May., 53	58	37		May, 53	23	15
	Jul., 53	72	68		Jul., 53	6	2
	Nov./Dec., 53	72	26		Nov./Dec., 53	11	20
	Apr., 54	78	60		Apr., 54	9	16
	Aug., 54	91	57		Aug., 54	Trace	3
	Nov., 54	85	37		Nov., 54	1	15
	Apr., 55	88	31		Apr., 55	5	30
	Aug., 55	83	43		Aug., 55	3	18
	Apr., 56	50	—		Apr., 56	24	—
	Aug., 56	—	26		Aug., 56	—	27
Cocksfoot	Jul., 50	6	26	Other species	Jul., 50	2	6
	Oct., 50	2	4		Oct., 50	2	Nil
	Aug., 51	1	11		Aug., 51	2	10
	Oct., 51	2	—		Oct., 51	3	—
	Apr./May, 52	2	5		Apr./May, 52	6	33
	Nov., 52	3	17		Nov., 52	8	14
	Jan./Feb., 53	2	7		Jan./Feb., 53	18	12
	May, 53	2	10		May, 53	14	2
	Jul., 53	1	6		Jul., 53	10	Nil
	Nov./Dec., 53	1	13		Nov./Dec., 53	10	6
	Apr., 54	1	17		Apr., 54	10	1
	Aug., 54	Trace	7		Aug., 54	Trace	3
	Nov., 54	Trace	5		Nov., 54	2	17
	Apr., 55	1	14		Apr., 55	4	19
	Aug., 55	Trace	12		Aug., 55	8	4
	Apr., 56	4	—		Apr., 56	15	—
	Aug., 56	—	11		Aug., 56	—	21
Other grasses	Jul., 50	4	10				
	Oct., 50	9	17				
	Aug., 51	2	24				
	Oct., 51	6	—				
	Apr./May, 52	2	Trace				
	Nov., 52	5	28				
	Jan./Feb., 53	2	4				
	May, 53	3	34				
	Jul., 53	10	24				
	Nov./Dec., 53	5	35				
	Apr., 54	2	6				
	Aug., 54	8	30				
	Nov., 54	12	26				
	Apr., 55	2	6				
	Aug., 55	6	23				
	Apr., 56	7	—				
	Aug., 56	—	15				

TABLE 14. LONG-TERM COMPARISONS: "CAGE" AND "MOWING AND CLIPPINGS RETURNED"

MEAN PERCENTAGES OF SPECIES IN MOWN HERBAGE ON CONTROL PLOTS

Species	Date Dissected	Technique		Species	Date Dissected	Technique	
		Cage	M.C.			Cage	M.C.
Perennial ryegrass	Jul., 50	74	40	White clover	Jul., 50	14	31
	Oct., 50	62	50		Oct., 50	16	20
	Aug., 51	68	34		Aug., 51	18	24
	Oct., 51	71	—		Oct., 51	12	—
	Apr./May, 52	61	4		Apr./May, 52	21	38
	Nov., 52	63	14		Nov., 52	14	17
	Jan./Feb., 53	44	4		Jan./Feb., 53	28	27
	May, 53	62	45		May, 53	17	5
	Jul., 53	76	64		Jul., 53	6	1
	Nov./Dec., 53	77	30		Nov./Dec., 53	6	6
	Apr., 54	66	51		Apr., 54	10	6
	Aug., 54	92	52		Aug., 54	Trace	3
	Nov., 54	83	41		Nov., 54	1	7
	Apr., 55	90	37		Apr., 55	4	14
	Aug., 55	74	55		Aug., 55	2	10
	Apr., 56	50	—		Apr., 56	15	—
	Aug., 56	—	—		Aug., 56	—	—
Cocksfoot	Jul., 50	6	16	Other species	Jul., 50	2	7
	Oct., 50	2	14		Oct., 50	13	2
	Aug., 51	3	11		Aug., 51	6	7
	Oct., 51	1	—		Oct., 51	8	—
	Apr./May, 52	3	8		Apr./May, 52	8	47
	Nov., 52	3	12		Nov., 52	10	35
	Jan./Feb., 53	2	7		Jan./Feb., 53	15	49
	May, 53	2	15		May, 53	8	3
	Jul., 53	1	10		Jul., 53	3	1
	Nov./Dec., 53	1	6		Nov./Dec., 53	5	12
	Apr., 54	1	25		Apr., 54	10	4
	Aug., 54	Trace	12		Aug., 54	Trace	3
	Nov., 54	Trace	6		Nov., 54	Trace	18
	Apr., 55	1	16		Apr., 55	4	29
	Aug., 55	2	11		Aug., 55	6	7
	Apr., 56	2	—		Apr., 56	18	—
	Aug., 56	—	—		Aug., 56	—	—
Other grasses	Jul., 50	4	6				
	Oct., 50	7	14				
	Aug., 51	5	24				
	Oct., 51	8	—				
	Apr./May, 52	7	3				
	Nov., 52	10	22				
	Jan./Feb., 53	11	13				
	May, 53	11	32				
	Jul., 53	14	24				
	Nov./Dec., 53	11	46				
	Apr., 54	13	14				
	Aug., 54	7	30				
	Nov., 54	16	28				
	Apr., 55	1	4				
	Aug., 55	16	17				
	Apr., 56	15	—				
	Aug., 56	—	—				

normal procedure in pasture management, it would seem that success has been achieved in maintaining a high quality pasture in the absence of stock under "mowing and clippings returned" management.

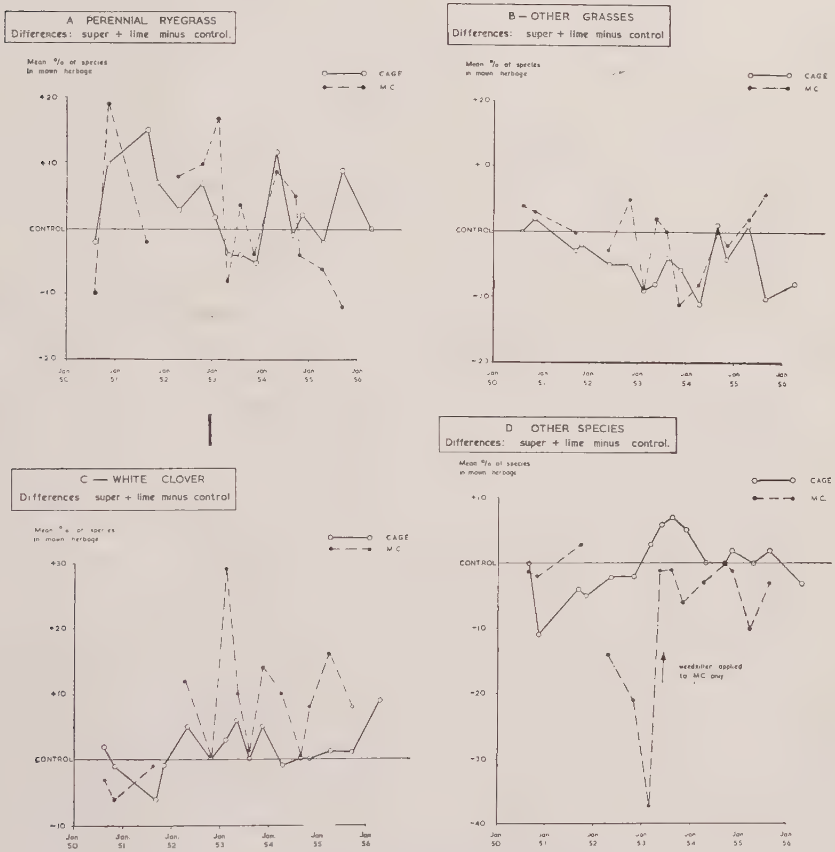


Fig. 3.—Effects of "cage" and of "mowings and clippings returned" techniques on botanical composition.

In Fig. 3 the effect of fertiliser (superphosphate plus lime) on the sward under the two techniques ("cage" and "mowing and clippings returned") is examined. Fig. 3D shows the effect of the weedkiller treatment on the weeds in the "control" plots. Without this spray treatment it seems likely that the deterioration of the sward in the "mowing and clippings returned" technique must have soon resulted in a serious decline in sward production especially on "control" plots. The effect of this would have been to increase the apparent response to fertilisers under this technique. This has been a disturbing influence to the trial. It is reasonable, however, to treat trials with weedkillers when the weeds that are present are largely the result of the technique employed and do not reflect a condition that would arise under "normal" grazing management.

The effect of superphosphate plus lime compared with control on the various pasture species has been as follows:—

Perennial Ryegrass. In most years except 1953 (and 1955 for the "mowing and clippings returned" technique a small but highly variable response to treatment is shown. With some exceptions this response is of a similar order under the two techniques.

Other Grasses. These have steadily increased on the control plots until mid 1954 when for some reason the difference between the contents of such species on control and superphosphate plus lime disappears for about a year. The "mowing and clippings returned" technique shows much greater variability with regard to this effect of treatment.

Other Species. "Mowing and clippings returned" technique seems to allow the ingress of weeds, particularly on the control plots, to a greater degree than does the "cage" method. These were readily controlled with weedkiller. The slightly greater amounts of weeds on the superphosphate plus lime treatment compared with control in the cage technique since 1953 seem to have been associated with an ingress of docks. These species are unpalatable to sheep and are particularly troublesome on areas of high fertility.

White Clover. The response of white clover to fertiliser treatment has been much more pronounced on the "mowing and clippings returned" technique than on the "cage" technique. There is a marked seasonal fluctuation in the amount of white clover which is at a minimum in the spring months.

The dissection of mown herbage gives an underestimate in terms of ground cover of low-growing species. Such species have a greater proportion of their leaf below the height of cutting than do more productive, erect plants. This effect tends to give overestimates of the amounts of species like perennial ryegrass and underestimates in terms of sward density of "other grasses" and "other species" (which are more prostrate and of lower productivity).

"Sears" and "Mowing and Clippings Returned" Techniques. Table 15 gives the sward dissection data that are available from the trials under these two techniques. From the beginning of the period involved, the sward of the "mowing and clippings returned" technique has had less perennial ryegrass and in most cases more cocksfoot, "other grasses" and "other species" than that of the "Sears" technique. After the spring of 1951, the white-clover content of both areas has been comparable.

In both techniques there is no evidence of progressive sward deterioration but both trials were sprayed with weedkiller in autumn 1953 as indicated previously. Marked seasonal fluctuations in the proportions of grass to clover occur with both techniques.

Effects of Techniques on the Chemical Analyses of the Soil

Table 16 gives the "quick test" chemical analyses for samples taken to 0–3 in. depth on this series of trials. Potassium is shown in parts

TABLE 15. "SEARS" v. "MOWING AND CLIPPINGS RETURNED" SECOND PERIOD 1951-53

MEAN PERCENTAGES OF SPECIES IN MOWN HERBAGE ON SUPER + LIME PLOTS

Species	Date Dissected	Technique	
		Sears	M.C.
Perennial Ryegrass	Jul., 51	73	26
	Aug., 51	—	32
	Oct., 51	84	—
	Apr., 52	33	12
	Nov., 52	57	39
	Feb., 53	25	25
	May, 53	86	57
	Jul., 53	87	64
	Dec., 53	60	38
Cocksfoot	Jul., 51	5	22
	Aug., 51	—	11
	Oct., 51	2	—
	Apr., 52	4	5
	Nov., 52	7	12
	Feb., 53	1	2
	May, 53	3	7
	Jul., 53	1	10
	Dec., 53	3	6
Other Grasses (mainly Yorkshire Fog and *Poa annua*)	Jul., 51	7	23
	Aug., 51	—	24
	Oct., 51	7	—
	Apr., 52	Trace	Trace
	Nov., 52	12	23
	Feb., 53	6	11
	May, 53	Nil	27
	Jul., 53	9	24
	Dec., 53	13	32
White clover	Jul., 51	9	24
	Aug., 51	—	23
	Oct., 51	7	—
	Apr., 52	50	50
	Nov., 52	18	14
	Feb., 53	46	39
	May, 53	10	8
	Jul., 53	2	1
	Dec., 53	19	18
Other species	Jul., 51	6	5
	Aug., 51	—	10
	Oct., 51	Trace	—
	Apr., 52	13	33
	Nov., 52	6	12
	Feb., 53	22	23
	May, 53	1	1
	Jul., 53	1	1
	Dec., 53	5	6

of K per 200,000 of soil extract (Morgans and phosphorus in parts P per 50,000,000 of soil extract (Truog).

TABLE 16. QUICK-TEST SOIL ANALYSES OF TRIALS (0–3 in.)

Technique	Date of Sampling	pH			K			P		
		Control	Super	Super + L	Control	Super	Super + L	Control	Super	Super + L
Sears	20/2/53	5.6	5.6	6.3	V.H.	V.H.	V.H.	3	9	10
M.C.	27/2/53	5.7	5.7	6.4	V.H.	V.H.	V.H.	2	7	9
	23/3/54	5.6	5.6	6.3	18	16	17	2	8	10
	15/3/55	5.6	5.6	6.3	17	13	16	3	10	9
	6/4/56	5.7	5.7	6.4	13	8	12	2	8	8
Cage	17/11/53	5.6	5.4	6.3	26	22	29	2	9	14
	15/3/54	5.3	5.4	6.1	26	22	26	4	11	13
	2/6/54	5.6	5.6	6.2	26	23	26	3	12	15
	10/8/54	5.4	5.4	6.4	23	23	27	4	13	17
	15/9/54	5.6	5.6	6.3	28	23	27	3	15	16
	26/3/56	5.4	5.3	6.2	24	24	28	3	12	18
	18/7/56	5.4	5.5	6.2	24	22	24	3	16	22
	23/1/57	5.6	5.6	6.4	21	23	26	3	17	20

V.H. = Very High: System used before introduction of numerical scale. In general, figures over 10 to 12 for both K and P are in the "high" range.

These analyses may be summarised as follows:

1. No evidence of build-up of control plots in respect of either pH or P on either "mowing and clippings returned" or on "cage" technique during the progress of the trial.

2. On "mowing and clippings returned" technique the pH and the P figures for superphosphate and superphosphate plus lime treatments remain constant. There is evidence of a decline in K levels however which, if continued, might soon be at a stage when potassium deficiency would occur.

3. On the "cage" technique, the pH and the K figures are relatively constant on all treatments but there is some tendency for an increase in P values on phosphate-treated areas. This increase is more marked in the presence of lime, but the lime plots have had higher P tests since the first analyses were made.

The techniques differ, therefore, in that there is a build-up of P figures on the "cage" method which is not shown in the "mowing and clippings returned" phosphate treated plots, and the latter trial shows a general decline in K test not seen in the "cage" technique area. This decline might be associated with a loss of K in grass clippings which are occasionally discarded when growth is very rapid. Excessive amounts of herbage returned to the plots will damage the sward. It seems that the potassium status of trials under "mowing and clippings returned" technique must be closely watched.

Labour and Land Requirements of Techniques

These have been classified as follows:

Technique	Land Required	Labour Required
Cage	Large areas: generally more than one acre per trial. With sheep a plot size of about 1/10th acre is minimum. Adequate replication is difficult. Costly in fencing, water supply, etc.	Skilled technical labour needed for both pasture and stock management. Pasture measurements are not laborious but stock production data are time-consuming to secure.
Mowing and Cippings Returned	Small-plot trial, usually about ½ acre. Adequate replication easy to achieve. Cheap.	Cutting and returning clippings should not take more than ½ day for 2 men. Little equipment needed. Can be managed at a distance from a research station.
Mowing and Grazing (Hudsons)	Small-plot trial with duplicate sections: requires about 1 acre.	Cutting is a little more laborious than "mowing and clippings returned". More work needed in control of sheep for grazing section not under mowing.
Proportional Return (Sears)	As for "mowing and clippings returned."	Heavy labour demands and unpleasant work involved. Must be on a research station with adequate technical labour and laboratory facilities.
White Clover	As for "mowing and clippings returned."	Cutting requires no more labour than for "mowing and clippings returned", but much time must be spent keeping the area weed-free.

DISCUSSION

This series of trials may seem to have been rather haphazardly designed but it represents a series of investigations over a considerable period. When techniques were shown to have serious drawbacks of one sort or another, they were withdrawn from the series and others were introduced. In the final analysis the work led to the selection of a small-plot method which would give, in the most economic manner, results similar to that of a grazing trial under the "cage" technique.

A parallel investigation of various ways of handling this "cage" technique has been completed at the Marton Experimental Area (Lynch and Mountier 1954) and this trial confirmed the advantages of the "Standard" Method of the Extension Division which was the one adopted in this case. The "cage" technique has therefore been considered to be the standard against which other techniques are compared. For trials where stock grazing is an essential feature and where stock production data are required the authors consider this "cage" technique to be the most reliable and efficient method available. Because of the high labour and land requirements and high cost of running the trials under this technique, however, it was essential to devise a small-plot technique particularly suited for fertiliser experiments on pastures, that would fill the following requirements.

(a) Demand the least amount of technical labour and not require constant supervision.

(b) Be capable of being carried out in areas remote from a research station. It is essential to make soil fertility studies on soil types where they are found, unless pot experimental methods are used. It is likewise impossible to have research stations on all important soil types.

(c) Require relatively small areas of land and the minimum of fencing.

(d) If possible, avoid the use of stock. Where stock are carried labour requirements are high, costs of laying down and carrying out trials are heavy and it is difficult to make satisfactory arrangements with co-operating farmers.

(e) Give a measure of treatment response closely similar to that of the "cage" technique and with low errors of measurement.

(f) Enable the carrying-out of trials with a large number of variables.

It seems that the "mowing and clippings returned" technique meets these requirements. There are, however, several difficulties associated with this method.

1. Difficulties of returning clippings without damage to the sward. In periods of rapid growth in the spring, clippings may be so heavy as to smother the sward if returned to plots after weighing. The effect of discarding a high proportion of the clippings may, after a few seasons, seriously deplete mineral reserves, especially of potash, and probably also affect the nitrogen status of the soil. Investigations are now under way of methods of decomposing or of "liquidising" clippings so that return of all herbage mown may be possible under all conditions.

2. The absence of grazing may result in changes in sward composition. Weeds and grasses palatable to sheep compete more successfully in the absence than in the presence of the grazing animal. It may be necessary to use weedkillers to restore the normal balance to the sward. In some cases where control with weedkillers is not feasible, it may only be possible to conduct a trial under this technique for a few years.

3. The speed of return of nutrients through decomposition of the cut herbage may be different from that occurring under grazing. It is probable that the cycle of return of nitrogen and potash does differ

from natural grazing conditions and the importance of this is hard to estimate. For phosphate, calcium, and nitrogen, this series of trials indicates, however, that any effect of this nature has not seriously affected responses to fertiliser treatments.

4. The method would have application only in humid areas and probably only where the worm population was sufficiently high to ensure rapid incorporation of cut herbage in the soil. It has been successfully used in a trial under sprinkler irrigation, however. If success is achieved in devising ways and means of decomposing or otherwise pre-treating cut herbage before return to the sward, it may be possible to use the technique in more arid areas and under dry seasonal conditions.

5. Herbage must be cut at a reasonably short stage of growth due to the difficulties of getting satisfactory decomposition of long growth without damage to the sward. Again if it is possible to decompose the herbage before return, this disadvantage may be overcome.

The other small-plot techniques compared in this series of trials seem to measure differences between the effects of fertiliser treatments of a similar order to the "mowing and clippings returned" technique and have no real advantages over that method. Thus the "mowing and grazing" technique requires the use of stock and also suffers from the disadvantage of having a series of plots of different treatments being grazed in a common enclosure. The "white clover" technique, despite earlier promising results, does not seem to be as sensitive as a mixed sward and it is most difficult to establish and maintain a pasture dominantly white clover. Sears' proportional return method is most laborious to conduct and can be carried out only on research areas.

For the foregoing reasons and from the evidence that has been given in this paper, the Extension Division has adopted the "mowing and clippings returned" technique for most soil-fertility investigations in New Zealand. Some 150 trials under this technique are now in progress, and the results that are becoming available from these trials support the conclusions that were reached in this investigation.

References

Hudson, A. W. 1933: *I.A.B. Herb. Publ. Ser. Bull. 11:* 21.
Lynch, P. B. 1947: *N.Z. J. Sci. Tech. A28:* 385.
Lynch, P. B.; Mountier, N. S. 1954: *Ibid. A36:* 375.
Sears, P. D. 1944: *Ibid. A25:* 177.

A QUICK METHOD FOR APPLYING INSECTICIDE DUST TO EXPERIMENTAL SMALL ANIMALS

By P. L. THOMAS* and B. O'GRADY, Animal Research Station,
Department of Agriculture, Wallaceville.

(*Received for publication, 13 March 1958*)

INTRODUCTION

The occurrence of external parasites such as fleas and lice in experimental small animal colonies occasionally causes some inconvenience. This is particularly true of the guinea pig which is frequently kept in very large numbers and which the lice *Gyropus ovalis* and *Gliricola porcelli* very successfully parasitise, being able to spread rapidly through a colony.

To dip or spray guinea pigs may prove fatal as these animals are very prone to chilling and, while it has been found possible to dip on still warm summer days without incurring any losses, it is impossible to do so during the cooler months. Dusting individual animals is time-consuming when there are large numbers to be treated.

Since the authors were associated with extensive tests of dusting machines for treating sheep, the idea of making a dusting machine for the treatment of guinea pigs naturally arose when it was discovered that the colony at this Research Station was infested with lice during a period when dipping could not be considered.

A prototype duster was designed by the writers and constructed with several structural improvements by the workshop staff. This model has now been used successfully on several hundred guinea pigs and its details are published here, together with comments on possible improvements, in order that others interested may develop their own machines.

CONSTRUCTIONAL DETAILS

The structural details of the machine are shown in Fig. 1.

Basically, there is a dusting chute four inches wide, three feet long and one foot deep made of galvanised iron. The general angle of inclination of the chute is 20° which is increased to 70° at the entry.

Dust is blown into the race from four nozzles, one on each side, one on the top and another in the floor. The last is covered with fine mesh but otherwise the outlets are open slit orifices. A $\frac{3}{8}$ inch diam. polythene tube leads from each of the four nozzles to a junction point fixed on one side of the race, which also serves as the point attachment for the main 1-inch hose connected to the dust blower.

The blower used was a Sturtevant 230 volt 550 watt electric blower, into the air intake of which, powder was fed from a pipe

---

* Present address: Tasman Vaccine Laboratory Ltd., Upper Hutt.

N.Z. J. agric. Res. 1: 522–524.

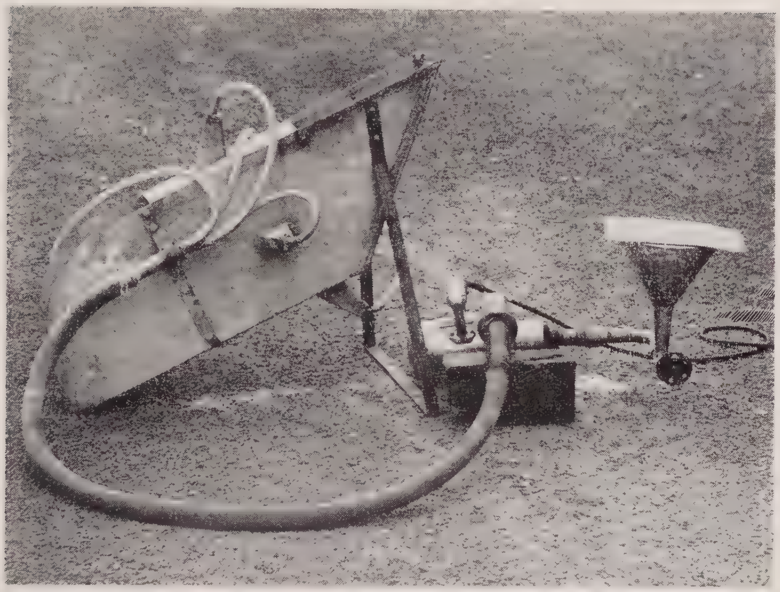


Fig. 1.—Apparatus for applying dust to small animals.

connected to a hopper (an 8-inch diameter funnel) and an air inlet. Provided that a reasonably air-tight cover was put on the funnel, the powder flow could be regulated by altering the inflow of air through the air intake by means of a regulating disc punched with various-sized holes. As the air inflow was reduced, more suction was exerted on to the powder which was then sucked from the hopper in quantities, in addition to that falling by gravitational effect through the $\frac{1}{4}$-inch diameter hole from the funnel into the pipe.

Dust

The only dust used to date has been a free-flowing marble dust with 3% dieldrin. The use of this preparation was dictated solely by the fact that some was readily available and there is no suggestion that this insecticide is the only one suitable or that the strength used is optimal, although there is no doubt as to its efficiency.

Method of Operation

The guinea pigs are gathered into a corner of an outside pen using a hinged board to keep them confined. The machine is set running and the animals are put head first into the chute. The speed of the operation is governed only by the speed at whch the guinea pigs can be picked up and put into the chute.

Thirty animals are normally put through the chute in each minute using approximately 2.5 g of dust per animal. After treatment the animals are let back into their indoor pens and the outside yard is washed down.

Using this system there have been no casualties. If the method were used indoors it would be advisable for food and water hoppers to be removed and litter removed from the floor of the pens.

In all cases it is advisable that the operators should wear simple dust-filtering masks.

Modifications

Two modifications are now thought to be worthy of consideration after use of the machine with quite a large number of animals.

The first is that the entrance should be funnel shaped with a wider aperture so that the animals can be slid in more easily, as with the present opening occasional animals will stretch out and hold on to the sides, spanning the aperture with their bodies and slowing down operations while they are dislodged and put in correctly.

The second is that the slope of the race should be altered to a curve in the manner of the slides to be found in children's playgrounds, so that the angle of fall is greatest in the middle and less at the end. The exit end should also be modified so that there is a slight drop when the animals leave the race. At present a few try to crawl back up the race. This has been overcome with the present model by putting a 2 inch block under the exit lip of the chute and blocking up the back support in the same way to maintain the correct angle of fall.

ACKNOWLEDGEMENTS

Mr. G. Hamlin and Mr. H. Sawtell constructed the machine and Mr. A. Bryant prepared the figure.

DAMAGE BY CLOVER CASE-BEARER CATERPILLARS

By J. M. KELSEY, Entomology Division, Department of Scientific and Industrial Research, Ashburton.

(*Received for publication, 4 March 1958*)

Summary

Results of inspections of flowers from nine clover paddocks in Ashburton County for injury by *Coleophora* spp. are given. The damaged seed represented nearly 63% and 8% of all seeds setting in white and red clovers respectively. The first cases of *Coleophora* damage to red-clover seed in New Zealand are recorded.

INTRODUCTION

For some years past, farmers in Canterbury had been reporting severe damage to white-clover seed by *Coleophora* spp. caterpillars and the present work was undertaken to secure basic information on severity of damage in relation to seed yields. Areas selected were taken at random from those intended for seed harvesting during 1955, and results should give a reasonable guide to overall damage in Ashburton County.

TECHNIQUE

Seven paddocks of white clover and two of red clover were examined. Sampling was based on a systematic pattern, and consisted in collecting 10 mature flower heads in an area of 1 square foot at each of 50 sampling points spaced at 20-pace intervals in four equally spaced rows dividing each paddock into approximate fifths. There were thirteen sampling points in each of the first and last rows and twelve in each of the intervening rows. Thus 500 flower heads were taken from each paddock. Samples were collected during the second and third weeks of February 1955.

All flower heads were examined and dissected under either the 7× or 20× microscope. In all, 3,500 white-clover and 1,000 red-clover flower heads were examined, and only seeds with live larvae present were recorded as damaged by case-bearer caterpillars.

WHITE CLOVER (*Trifolium repens* L.)

At the time of the survey there was, for the seven paddocks, an average of 54, and a range of 49–69 florets per flower head for white clover, and the average number of florets that set seed was 48 with a range of 47–51. Seeds setting per floret averaged 3.6 with a range of 1–5. These averages gave a figure of 172.8 seeds per flower head for a crop not infested by case-bearer caterpillars. From the table it will be seen that for 96 acres the average reduction in seed yield of white clover due to case-bearer damage was 62.85% and that only one of the seven paddocks had no caterpillars recorded. All paddocks were 2 or 3 years from sowing down, and most damage occurred to the third-year clover stands (the first 5 in Table 1).

RED CLOVER (*Trifolium pratense* L.)
(Montgomery strain)

Examination of florets from red clover showed that case-bearer caterpillars caused an average reduction in seed yield of 7.92% in two paddocks totalling 58 acres. In this instance there was only one seed per floret, and a high percentage of florets that did not set seed at all: there was a range of 82–111 florets per flower head, but the average number that set seed was only 60.

Though damage to red clovers by *Coleophora* spp. has been recorded in overseas literature, this is the first record of these caterpillars injuring red-clover seed in New Zealand.

TABLE 1. CLOVER SEED YIELD: DATA BASED ON 500 FLOWER HEADS FROM EACH PADDOCK

District	Area (acres)	Av. No. Good Seeds per Flower Head	% Seed Reduction	Av. No. Florets per Flower Head	Av. No. Florets Setting Seed
			White Clover		
Wakanui	3	3.4	97.95	52	46
Wakanui	10	26.2	84.84	54	48
Highbank	20	28.6	83.45	54	48
Coldstream	15	28.8	83.34	56	48
Highbank	12	29.7	82.45	53	47
Lauriston	14	169.0	7.96	54	51
Ealing	22	172.8	Nil	55	48
	96	65.5	62.85	54	48
			Red Clover		
Ealing	27	43.3	10.95	95	59.8
Lagmhor	31	57.95	4.9	98	61.0
	58	50.62	7.92	96.5	60.4

RACES OF POTATO BLIGHT, *Phytophthora infestans* (Mont.) de Bary, IN NEW ZEALAND

By C. M. DRIVER, Crop Research Division, Department of Scientific and Industrial Research, Lincoln.

(*Received for publication, 10 March 1958*)

Summary

A small survey of blight races present in New Zealand was conducted in 1956-57.

The common race 0 was present in several commercial crops in different regions, and was found also on the native species *Solanum aviculare* from Auckland and *S. laciniatum* from Lincoln. On a crop near Dunedin only race 4 was found. Races 2; 4 and 2,4 were found in breeding plots at Lincoln. In similar plots at Otahuhu, races 2 and 4 were found at an earlier collection and races 2; 3; 4; 1,3; and 2,4 at a later date.

It is suggested that in view of the probable genetic constitution of the races found, there is a strong possibility of all races occurring now or in the future in New Zealand, and a more extensive survey would show many races to be widespread in commercial crops.

INTRODUCTION

Immunity to potato blight caused by the fungus *Phytophthora infestans* (Mont.) de Bary, was found in the wild Mexican species *Solanum demissum* Lindl. in 1909 (Salaman 1910). By crossing to commercial varieties, some useful immune hybrids were produced, but in 1932 a new race of blight appeared to which some of the hybrids were susceptible (Salaman 1949). Breeding for resistance has continued, but, as resistant genotypes have been produced, new races of blight have arisen to attack them. Not only do new races become established with the production of resistant genotypes, but they can be present in non-resistant commercial crops and in some cases one of the new races appears to have become dominant (e.g. race 4 in Ireland—Doling 1956).

It is now known that in *S. demissum* there are four (and possibly five) genes each giving resistance to different groups of races. These genes have been named R_1, R_2, R_3, and R_4 (Black 1952). The races of blight have been named in a corresponding manner (Black *et al.* 1953). For example, race 0 is unable to attack any genotype with a resistance gene; race 1 can attack genotype R_1 but not R_2, R_3 or R_4; race 1,2 can attack genotype R_1R_2 but not those with either or both R_3 and R_4; race, 1,2,3,4 can attack all resistant genotypes. Using this nomenclature races can be postulated which will attack any resistant genotype, and so far all except one race have been found in the field. Consequently breeding for race resistance is of doubtful value.

In New Zealand, breeding for blight resistance has utilised the genes R_1 to R_4 which give resistance through a hypersensitive reaction.

Much material bred for resistance to the common race has succumbed in the field, and although some hybrids have remained immune, it would be desirable to know if their resistance is likely to be permanent. Consequently, a check on races present in New Zealand was thought desirable. If few races were present and the new races could occur only after introduction from another country, breeding for resistance to those present could be of value. On the other hand, if many races were present or new races could arise by segregation or mutation from the common race, then any resistant hybrid relying on race resistance would be bound to succumb ultimately to a new race.

METHODS

Samples of blight-infected leaves or tubers were obtained from the sources listed in Table 1. Spore suspensions prepared by inoculating each sample to the susceptible variety, Chippewa, were inoculated on to the series of differentials given by Black *et al.* (1953), in order to differentiate races according to the "International System of Nomenclature" (*Ibid.*).

RESULTS

Races were differentiated as shown in Table 1.

TABLE 1. RACES OF POTATO BLIGHT FOUND IN NEW ZEALAND

Plant Source	Source	Area Collected	Date Collected	Races Found
Potato hybrids	Veg. Res. Sta.	Otahuhu, Auck.	Nov. 1956	2; 4
Potato hybrids	Veg. Res. Sta.	Otahuhu, Auck.	May, 1957	2; 3; 4; 1, 3; 2, 4
Arran Chief	Dep. Agric.	Auckland	May, 1957	0
*S. aviculare**	Plant Dis. Div.	Auckland	June, 1956	0
*S. laciniatum**	Linc. Domain	Lincoln, Canty.	June, 1957	0
Dakota Red	Crop Res. Div.	Lincoln, Canty.	May, 1957	2; 4; 2, 4
Auckland Short Top	Crop Res. Div.	Lincoln, Canty.	May, 1957	0
Dakota Red	Dr. I. D. Blair, Lincoln Coll.	Sthbridge, Canty.	May, 1957	0
Potato hybrids	Exp. plots	Greendale, Canty.	May, 1957	0
Black's hybrid 1563c (14)	Exp. plots	Greendale, Canty.	May, 1957	4
King Edward	Dep. Agric.	Kerrytown, S.C.	June, 1957	0
Arran Chief	,, ,,	Waimate, S.C.	June, 1957	0; 1
—	,, ,,	Mosgiel, Otago	June, 1957	4
King Edward	,, ,,	Ingill, Southland	July, 1957	0
King Edward	,, ,,	Tuatapere, Sthlnd	July, 1957	0
King Edward	,, ,,	Tuatapere, Sthlnd	July, 1957	0

* (Driver 1957).

DISCUSSION

Race 0 appears to be the most common race in New Zealand, though race 4 is apparently common throughout the country as well.

This is in line with observations in Ireland (Doling 1956) and Canada (Graham 1955), and it will be interesting to see if race 4 becomes dominant here as it appears to be becoming in those and some European countries.

Only relatively small samples were collected from each crop, yet four out of sixteen isolates proved to be mixtures. This suggests that more detailed collections would reveal the presence of a much greater range of races within each crop. In this first survey were found races 1; 2; 3; 4, which are able individually to overcome the resistance conferred by the individual genes R_1, R_2, R_3, and R_4. Thus it would seem that the genes are already present in the fungal population from which any of the races present overseas could be synthesised, up to, and including, the master race 1, 2, 3, 4 capable of attacking the most resistant genotype $R_1R_2R_3R_4$.

The greatest range of races (5) was found on the breeding plots at Otahuhu where blight is present throughout the year, and a smaller range (3) was found on the breeding plots at Lincoln where blight occurs in autumn in wet seasons only. It would seem that if we continue to breed for resistance to specific races, new races capable of attacking the resistant hybrids will arise, as has occurred in France over the past few years (Madec *et al.* 1956). For a more useful permanent effect it would seem more profitable to turn to field resistance, a non-specific reaction which is less likely to break down with the occurrence of new blight races. Already considerable progress has been made with this approach overseas (Niederhauser 1956; Mills *et al.* 1957), and highly field-resistant hybrids have been imported into New Zealand for use in a similar programme here.

ACKNOWLEDGEMENTS

Thanks are due to the officers of Plant Diseases Division and the Vegetable Research Station, Department of Scientific and Industrial Research, and of the Department of Agriculture for collecting potato-blight isolates, and to Mr. H. W. Findon for technical assistance.

REFERENCES

BLACK, W. 1952: A Genetical Basis for the Classification of Strains of *Phytophthora infestans*. *Proc. roy. Soc. Edin. B65:* 36-51.

BLACK, W.; MASTENBROEK, C.; MILLS, W. R.; PETERSON, L. C. 1953: A Proposal for an International Nomenclature of Races of *Phytophthora infestans* and of Genes Controlling Immunity in *Solanum demissum* Derivatives. *Euphytica 2:* 173-8.

DOLING, D. A. 1956: Distribution of Physiological Races of *Phytophthora infestans* (Mont.) de Bary in Northern Ireland. *Nature 177 (4501):* 230.

DRIVER, C. M. 1957: Infection of Native *Solanum* Species by the Potato Blight Fungus. *Nature, Lond. 180 (4598):* 1367-8.

GRAHAM, K. M. 1955: Distribution of Physiological Races of *Phytophthora infestans* (Mont.) de Bary in Canada. *Amer. Potato J. 32 (8):* 277-82.

MADEC, P.; PERENNEC, P.; LAISSUS, R. 1956: Travaux Effectueés Pendant la Période 1945-55. *Ann. Inst. nat. Rech. agron., Paris 6 (3B):* 319-82.

MILLS, W. R.; NIEDERHAUSER, JOHN S.; HOUGAS, R. W. 1957: Blight Resistance of Selected *Solanum* Hybrids in Mexico. *Phytopathology 47:* 25.

NIEDERHAUSER, JOHN S. 1956: The Blight, the Blighter, and the Blighted. *Trans. N.Y. Acad. Sci., Ser. II 19 (1):* 55-63.

SALAMAN, R. N. 1910: The Inheritance of Colour and Other Characters in the Potato. *J. Genet. 1:* 7-46.

————1949: "History and Social Influence of the Potato." p. 178. C.U.P.

THE USE OF CONDENSED WHEY AS A FOOD FOR FATTENING PIGS

By A. C. DUNKIN, Massey Agricultural College, Palmerston North.

(*Received for publication, 21 March 1958*)

Summary

A trial with fattening pigs is reported concerning the feeding value of condensed whey (60% D.M. content) when fed in conjunction with different fixed quantities of meal.

The results of a preliminary trial indicated that it was unnecessary to dilute condensed whey before feeding when drinking water was provided.

In a subsequent trial, three rations containing fixed daily meal allowances of 2 lb, 1 lb, and ½ lb plus condensed whey were compared with an all-meal ration over the live-weight range 53–110 lb.

For the overall trial period condensed whey constituted 40%, 69%, and 82% of total D.M. intake respectively of the groups receiving 2 lb, 1 lb, and ½ lb meal daily. No scouring occurred on any treatment. Relative to that of the control group, growth rates of the whey groups arranged in descending order of meal allowances were 99%, 80%, and 72% respectively. The corresponding figures for efficiency of food utilisation were 94%, 79%, and 72%. In respect of both growth rate and food economy, the all-meal and 2 lb meal rations were significantly superior to the two lower meal levels ($P < .01$).

Although comparing unfavourably with the all-meal group, condensed whey supplemented with only ½ lb meal daily permitted a weekly gain of 6.1 lb, which is very satisfactory by New Zealand standards for fresh whey feeding.

Estimates of the amount of condensed whey required to replace 1 lb barley meal varied from 1.5 lb to approximately 2 lb, depending on the level of supplemental meal.

The economics of whey concentration under New Zealand conditions are discussed briefly in relation to these results.

INTRODUCTION

In spite of the fact that whey is second only in importance to skim milk as a basic food in New Zealand pig production, there is little critical information regarding its use under the rather specialised conditions obtaining in this country.

Experimental work is in progress with the broad objective of securing information regarding the utilisation of whey by fattening pigs fed limited amounts of meal supplements. For some aspects of this work it has been found convenient to use a concentrated whey, so that the nutritional value of whey solids can be studied without the complication of excessive bulk; conversely, the effect of varying degrees of dilution of the material on food intake and efficiency of utilisation can be observed.

Apart from these considerations, owing to the highly seasonal character of New Zealand dairying, large quantities of whey run to waste during the peak months of production, while, during the winter, supplies of fresh dairy by-products for stock feeding drop to negligible proportions. In these circumstances the possibility of conserving surplus whey by concentration for use when fresh supplies are short has particular appeal. However, before the economic feasibility of such a proposal could be assessed, data were required concerning the feeding value of the material under New Zealand conditions.

The literature on the use of concentrated whey products for fattening pigs is restricted mainly to results of its use as a partial replacement of cereal supplements, both in all-meal rations and in combination with bulky foods in modified Lehmann feeding systems. This work was undertaken chiefly in Germany, Sweden, and the Netherlands, Bünger (1939) and Schmidt *et al.* (1939) reporting on the use of dried whey (approximately 90% D.M.) and Edin and Nordfeldt (1941) and De Man (1950) on the use of condensed whey (60-66% D.M.). Although De Man found that when condensed whey partially replaced ensiled steamed potatoes growth rate was improved by about 20%, in general there appears to be agreement that it is not possible to replace the entire cereal portion of the diet with whey without affecting growth rate adversely.

In contrast to the use of whey concentration products in relatively limited quantities, there is little information as to their value when comprising the major portion of the diet. In a small non-replicated trial reported by Scott and Graham (1929), by comparison with a group receiving condensed whey alone fed *ad libitum,* weaner pigs fed a daily supplement of $\frac{1}{2}$ lb meat meal showed improved growth rate up to 70 lb live weight but not thereafter. For the initial phase of the trial, when the pigs were confined on concrete, they would only eat the condensed whey in a diluted form and it was therefore fed diluted 1:7 with water. Subsequently, however, when allowed access to pasture, the solid paste was eaten readily. Longwill (1948), in discussing the feeding of whey paste and mother liquor from which lactose had been extracted, also states that it is necessary to dilute these materials 1:9 and 1:5 respectively with water. It should be noted, however, that these materials have a higher concentration of soluble salts than ordinary condensed whey.

Since there are obvious disadvantages in feeding the material in an excessively dilute form, a preliminary trial was carried out in 1955 to check on this point. For a period of eight weeks, four pairs of pigs of approximately 90 lb initial weight were fed a similar basic meal allowance but their ration of condensed whey was diluted 1:1, 1:2, 1:3, and 1:8 with water. Drinking water was provided for all groups. No scouring occurred on any treatment, while the growth rate and food economy of all pairs were very similar and showed no obvious trend, suggesting that there is no need to dilute the condensed whey when adequate drinking water is available.

In the present paper are reported the results of a trial carried out in 1956, in which the feeding value of condensed whey, supplemented with different levels of meal, was compared with that of an all-meal ration.

Since this trial was completed, Becker *et al.* (1957) have reported on the effect of diets containing high levels of dried whey, derived from cheddar cheese production, for pigs at various stages of growth. Whereas levels of dried whey varying from none to 60% of a semi-synthetic diet fed *ad libitum* to baby pigs had no effect on growth rate and food economy, in the case of fattening pigs of 100 lb live weight, full-fed on similar diets, the 60% whey ration induced severe diarrhoea and markedly depressed food intake and growth rate. Some diarrhoea also occurred on the 40% whey ration, but growth was not significantly slower than that of the controls. In a subsequent group-feeding trial, substitution of 20% and 30% dried whey in a practical ration of corn and soya-bean meal fed *ad libitum* to weaning pigs seriously reduced food consumption and growth rate and produced a mild diarrhoea. It was suggested that the occurrence of adverse effects at appreciably lower levels of whey in this last trial might be connected with the differing mineral composition of the ration compared with that of the semi-synthetic rations.

METHOD

The experimental treatments consisted of condensed whey fed in conjunction with three different levels of meal and a fourth (control) treatment in which an all-meal ration was fed.

Animals Used and Experimental Design

Twenty-four Large White × (Large White × Berkshire) pigs were used, comprising six blocks each consisting of four litter mates matched for weight and previous growth rate. Intra-block allocation of pigs to the four experimental treatments was at random with the restriction that the treatment groups were balanced for sex. Initial live weights varied between blocks from 40–70 lb.

Condensed Whey

The material was light brown in colour and of a consistency rather thicker than treacle, which made it difficult to pour but not solid enough to be handled easily with a spade. It had a gritty texture, due to crystals of lactose, and a pronounced salty taste. The chemical analysis was as follows: Moisture 41.6%, protein 10.8%, lactose 37.8%, fat 0.7%, ash 7.2%, undetermined substances (citric acid, lactic acid, etc.) 1.9%. The NaCl content was 3.15% and the acidity was equivalent to 1.40% lactic acid. Samples taken for dry-matter determination

Treatment	Daily Meal Allowance	
I	½ lb meat meal	+ condensed whey diluted 1:1 with water
II	½ lb meal meal + ½ lb. barley meal	
III	½ lb meat meal + 1½ lb barley meal	
IV	½ lb meat meal + increasing amount of barley meal.	

from each drum as it was used showed little variability, ranging only from 59.9% to 60.8% D.M.

Experimental Treatments

The treatment meal allowances were as follows:

Since one of the aims of the trial was to obtain information on the amount of condensed whey that pigs could consume, a feeding scale was adopted which gave a daily D.M. intake 20% greater than that found by Smith (1956) to give maximum efficiency of food conversion on an all-skim milk diet over the live-weight range 40–80 lb. (See Table 1.)

TABLE 1. Feeding Scale.*

L. Wt. (lb)	D.M. Daily (lb)
41– 44	2.06
61– 64	2.58
81– 84	3.10
101–104	3.61
121–124	4.13

* Intermediate values obtained by interpolation.

Previous experience had shown that pigs take a few days to acquire a taste for the condensed whey. Accordingly this was introduced to all pigs eleven days before the trial began and all received 1½ lb meal daily and concentrated whey until the trial started. In order to prevent a sudden check occurring among the Treatment I pigs, their daily meal ration was reduced only to 1 lb for the first week of the trial (as for Treatment II), and thereafter to ½ lb over the following three days.

As the undiluted material is difficult to weigh out accurately, it was fed diluted 1:1 with water throughout the trial. In the case of Treatment IV (control) pigs, water was mixed with their meal in the ratio of 1 part meal to 3 parts water for the first five weeks and thereafter the ratio was reduced to 1:2.

Once weekly all pigs received 10 g of a Vitamin A and D_3 emulsion containing 5000 i.u. Vitamin A and 500 i.u. Vitamin D_3 per gramme.

General Management

The pigs were confined on concrete and were fed individually. Whey was fed three times daily, the meal allowance being given before the morning and afternoon feeds. All pigs had access to drinking water at all times. The pigs were weighed twice weekly prior to the morning feed and the rations adjusted according to the feeding scale. Observations on the consistency of the dung were made twice daily.

RESULTS

The mean live weight at the start of the trial was 53 lb, and the experimental treatments were continued until all pigs weighed at least 110 lb live weight. The mean growth curves for the four groups for the first eight weeks of the trial are shown in Fig. 1.

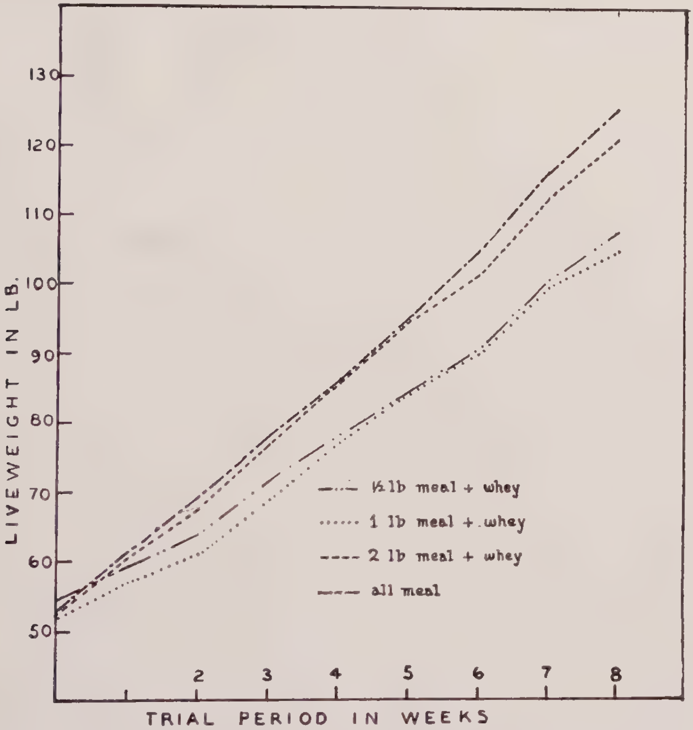


Fig. 1.—Average growth curves for each of the four experimental groups for the first eight weeks of the trial.

All pigs were in good health throughout the course of the trial. Dung was black and was consistently well formed in all groups receiving condensed whey. The only occasion that any scouring occurred was when replenishment of the water troughs was omitted inadvertently at one midday feed. When water was given at the afternoon feed, scouring ensued in the case of one pen of pigs which were allowed to gorge themselves.

Using covariance analysis as outlined by Snedecor (1946) to correct for the variable initial weight of the pigs, the treatment effects on growth were assessed in terms of the time taken to reach a standard live weight of 110 lb from the start of the trial.

A significant regression ($P < .01$) was obtained of time taken to reach 110 lb live weight on initial weight and the variance ratio of the adjusted mean squares was highly significant ($P. < .001$).

The mean times to reach 110 lb live weight, adjusted for differences in initial weight, were significantly greater for Treatments I and II than for Treatments III and IV, but differences between I and II and between III and IV were not significant. The adjusted mean times presented in Table 2 correspond to weekly live-weight gains of 6.1, 6.8, 8.4, and 8.5 lb for Treatments I to IV respectively.

TABLE 2. ADJUSTED MEAN TIMES FROM START OF TRIAL TO 110 lb LIVE WEIGHT.

Treatment	No. of Days	Significance of Differences
I	64.4	I v. II; III v. IV — N.S.
II	58.3	I, II v. III, IV $P < .01$
III	46.9	Error Coeff. Variation = 10.5%
IV	46.7	

Owing to the fact that only a communal supply of drinking water could be provided for each pen, each of which contained pigs on all four treatments, no data on the effects of the treatments on water consumption could be obtained.

Food refusals were small for all treatments. For the overall trial period, both Treatments I and II pigs left an average of 2% of condensed whey, the maximum individual refusal being 6%.

Efficiency of food utilisation data, expressed as total D.M. consumption from the start of the trial to 110 lb live weight, were analysed using covariance, with initial live weight as the independent variable. Results followed a similar trend to those for growth rate, both the regression and the variance ratio of the adjusted mean squares being significant. The error coefficient of variation was 9.0%. Food consumption and economy data, corrected for variation in initial weight, are presented in Table 3.

TABLE 3. ADJUSTED FOOD INTAKE AND EFFICIENCY OF FOOD UTILISATION FROM START OF TRIAL TO 110 lb LIVE WEIGHT.

	Treatment				Significance of Differences (Total D.M. only)
	I	II	III	IV	
Meal (lb)	38.4	58.2	94.1	156.6	
Condensed whey (lb)	253.6	193.4	93.3	—	I v. II; III v. IV —N.S. I, II v. III, IV — $P < .01$
Total D.M. (lb)	187.5	170.3	142.0	133.7	
lb meal/1 lb gain	0.68	1.03	1.66	2.76	
lb whey/1 lb gain	4.46	3.40	1.64	—	
lb D.M./1 lb gain	3.30	3.00	2.50	2.36	

Condensed whey formed 82%, 69%, and 40% of the total D.M. intake of Treatments I, II, and III respectively.

Whereas there was no significant difference in total D.M. intake between Treatments III and IV, Treatments I and II pigs required approximately 40% and 27% more D.M. respectively than the all-meal control group. The difference in intake between Treatments I and II fell just short of significance at the 5% level.

DISCUSSION

The dilution of condensed whey 1:1 with water resulted in a compulsory water intake that was less than the water consumption of fattening pigs of various weights as quoted by Davidson (1948): so that unless the saltiness of the material or some other characteristic resulted in a voluntary consumption of large additional quantities of water, the experimental results were not influenced by a possible difference in bulk between treatments.

The absence of scouring contrasted with the results reported by Scott and Graham (1929), and by Becker *et al.* (1957) in respect of finishing pigs receiving 40% or more of their ration as whey. It also contrasted with the frequent scouring that has occurred in our trials when fresh whey, supplemented with 1 lb meal daily, has been fed according to the scale given in Table 1. The fact that both Scott and Graham and Becker *et al.* used either *ad libitum* or full feeding as opposed to the rationing system adopted in our trial may account for this difference. However, this does not explain the discrepancy between the effects of fresh and condensed whey observed in our trials. The greater water content of fresh whey, its higher acidity and/or its day to day variability in these respects may be possible contributory factors and further information is being sought on this point. At all events, the results of the present trial indicate that young growing pigs can be fed condensed whey, supplemented with as little as ½ lb meal daily on a plane of feeding 20% higher than that recommended by Smith (1953) for fattening on skim milk without the occurrence of scouring or obvious adverse effects on health.

The fact that the average food refusals for Treatment I pigs were small and did not exceed those of Treatment II pigs shows that the level of feeding adopted was within the capacity of the pigs. In other words, in addition to ½ lb meal, it is possible for pigs of 50 lb live weight to consume at least 3.0 lb condensed whey daily, with this increasing to at least 5.5 lb at 110 lb live weight. Under commercial feeding conditions, where the pigs have constant access to the trough, these quantities could be exceeded.

The growth rate results agree with those reported by Becker *et al.* for finishing pigs to the extent that growth rate on Treatment I, in which whey represented 40% of total D.M. intake for the overall trial period, did not differ significantly from that on the control treatment, whereas higher whey levels seriously depressed rate of live-weight gain. Although growth was appreciably slower when the daily meal allowance fell below 2 lb, by comparison with that of pigs on typical New Zealand fresh-whey feeding, the growth rate of Treatment I pigs in particular was very satisfactory.

In view of the fact that pigs on all treatments had the same D.M. intake at any given weight, it was to be expected that efficiency of food utilisation for the different treatment groups would show a similar trend to that for growth rate. The results suggest that the growing pig is unable to make efficient use of whey solids when the daily meal allowance is less than approximately 2 lb. While there is a considerable volume of evidence to indicate that the high lactose content of whey is at least partly responsible for the decline in growth rate and food efficiency when whey constitutes too high a proportion of the ration, the beneficial effects obtained by Daniel and Harvey (1947) when dialysed whey was fed to rats suggests that the high soluble salt content of whey may also be implicated.

Some indication of the meal-replacement value of condensed whey may be obtained by equating the differences in barley meal consumption between the control group and the remaining three treatments to the condensed-whey consumption on the latter treatments. These comparisons are shown in Table 4.

TABLE 4. MEAL-REPLACEMENT VALUES OF CONDENSED WHEY FOR VARIOUS TREATMENT PAIRS

Treatment Comparison	lb Condensed Whey to Replace 1 lb Barley Meal	lb Whey D.M. to Replace 1 lb Barley Meal D.M.
IV v. III	1.49	1.06
IV v. II	1.97	1.39
IV v. I	2.15	1.52

As would be expected, the meal replacement value of the whey varies according to the proportion in the diet. If at least 2 lb meal is fed daily, it appears that condensed whey will replace barley meal practically weight for weight on a D.M. basis, whereas if 1 lb meal or less is fed, approximately 2 lb condensed whey is required to replace 1 lb barley meal.

Economic Considerations

Relating these ratios to the ruling price for barley meal, a rough estimate may be made of the maximum price that it is economic to pay for condensed whey. However, because of the problem of containers for transport and storage of condensed whey, and the greater difficulty of handling the material by comparison with meal, it is probable that in order to be an attractive proposition the price per ton of condensed whey would not have to exceed 40% of that of barley meal.

It must also be borne in mind that owing to the limiting effect on growth rate of high lactose intake, condensed whey will not replace barley meal as satisfactorily as the above ratios suggest if it is used as the only supplement to fresh whey. Its main application is therefore for use in periods when fresh dairy by-products are in short supply.

The cost of condensing surplus whey at cheese factories has been estimated at £14 to £18.10.0 per ton of condensed whey of 60% D.M. content, depending on the number of hours of operation of the plant and on how factory labour is organised (Mr. J. K. Scott, pers. comm.). These estimates include an allowance of £5 per ton to cover the capital costs of the necessary plant, but it has been assumed that whey concentration could be arranged in such a way that no increase in factory boiler capacity was required. If a concentration plant was already available, production costs would be appreciably less.

On the basis ~~of~~ the results obtained in this trial, present economic conditions in New Zealand are such that the suggested maximum price that the pig feeder would be prepared to pay for condensed whey is of a similar order to, or slightly less, than the estimated costs of concentration. However, in cases where cheese factories are faced with heavy expenditure in disposing of whey in a manner which does not create a river pollution or nuisance problem, these disposal charges should be deducted from the cost of whey concentration. In such circumstances the installation of a concentration plant could be as cheap a solution to the problem as any other, at the same time providing valuable out-of-season food for pigs.

ACKNOWLEDGEMENT

The author desires to thank Messrs. Dairy Products Ltd., Hawera, for the supplies of condensed whey used in this trial.

REFERENCES

BECKER, D. E.; TERRILL, S. W.; JENSEN, A. H.; HANSON, L. J. 1957: *J. Anim. Sci. 16:* 404.

BUNGER, H. 1939: *Tierernährung 11:* 276. Seen only in *Nutr. Abstr. Rev. 9:* 4995.

DANIEL, F. K.; HARVEY, E. H. 1947: *J. Nutr. 33:* 429.

DAVIDSON, H. R. 1948: "The Production and Marketing of Pigs". Longmans Green, London.

DE MAN, T. J. 1950: Nat. Co-op. Aan-en Verkoopvereniging voor de Landbrouw "Centraal Bureau" G.A. Rotterdam, Dec. 1950. See also *Nutr. Abstr. Rev. 20:* 6011.

EDIN, H.; NORDFELDT, S. 1941: *Lantbrukshogsk Husdjersförsok Rep. 5.* Seen only in *Nutr. Abstr. Rev. 15:* 2833.

LONGWILL, A. 1948: *N.Z. J. Agric. 76:* 187.

SCHMIDT, J.; KLIESCH, J.; SCHMALENBACH, H. 1939: *Z. Schweinez. 46:* 2. Seen only in *Nutr. Abstr. Rev. 9:* 1158.

SCOTT, M. J.; GRAHAM, R. W. 1929: *N.Z. J. Agric. 38:* 112.

SMITH, D. M. 1953: *N.Z. J. Agric. 86:* 125.

——— 1956: *N.Z. J. Sci. Tech. A38:* 217.

SNEDECOR, G. W. 1946: "Statistical Methods". Iowa State College Press, Iowa.

THE EFFECT OF LIGHT AND TEMPERATURE ON THE CHEMICAL COMPOSITION OF PASTURE PLANTS

By N. O. Bathurst, Plant Chemistry Division, and K. J. Mitchell, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 9 April 1958*)

Summary

The effect of different levels of light and temperature on the chemical composition of some representative pasture grasses and legumes was investigated. Substances estimated included various nitrogenous constituents, sugars, ash, fructosan, and organic acids.

The effect of two levels of light and of temperature has been measured. The most striking changes were found in nitrate content, this being highest with low light and high temperature and lowest with high light and low temperature. White clover and short-rotation ryegrass showed some differences in behaviour when compared with other species.

An experiment using white clover at three different levels of light, other conditions being similar, showed a definite relationship between light intensity and chemical composition.

Finally, results are reported for three species grown in controlled environment cabinets at temperature levels varying from 45°F to 95°F.

INTRODUCTION

Among the causes of differences in the chemical composition of plants of any one species, environmental factors play a very important part. Many plant constituents are subject to very rapid fluctuation in concentration with even comparatively slight changes in environment, yet our knowledge of the relationship between these changes is very incomplete.

During the course of an experiment on bloat in cattle (Johns 1955) an opportunity was taken to collect samples of ryegrass and clover from a mixed pasture over a period of 1½ years in the hope that analyses would show the effect on plant composition of seasonal changes, etc. The soluble nitrogenous constituents were found to vary, both qualitatively and quantitatively within quite short periods (24 hours), and no clear pattern could be detected when the results were examined over the whole period. It was felt, therefore, that in the present very incomplete state of our knowledge, it would be desirable to limit uncontrollable factors as far as possible and confine the investigation to plants grown under controlled conditions, with light and temperature being the only variables.

In this preliminary investigation, comparatively large differences of light and temperature were used. Most of the analyses performed were for groups of substances, e.g. total nitrogen, sugars, amino-acids,

N.Z. J. agric. Res. 1: 540–552.

ash, organic acids, fructosans, and carbohydrate, although nitrate and individual amino-acids were also measured.

EXPERIMENTAL

Plant Material and Growth Conditions

The first experiment was carried out in October-November 1954, under the different temperature conditions provided by growing some plants in a glasshouse and some outside, and the different conditions of illumination provided by growing some plants unshaded and some under scrim-covered frames which reduced the light to 28% of the unshaded value. Four series of plants, each of six pots, were grown: two in the glasshouse with and without shading (GL and GS series) and two outside, with and without shading (OL and OS series). The plants were grown in soil containing ample supplies of the usual nutrients, and whenever any deficiency of nitrogen seemed likely to occur it was supplied to all the pots at the same time in the form of sodium nitrate. In a repetition of this experiment the following spring, vermiculite watered with Hoagland's nutrient solution was used in place of soil.

In the second experiment three intensities of light were provided for white clover plants grown in pots of vermiculite watered with Hoagland's nutrient solution. One third of the pots were exposed to full light, one third were under frames covered with one layer of scrim reducing the light to 50% of that outside, and one third were covered with two layers of scrim, reducing the light to 28%.

For the third experiment the plants after establishment were grown in growth cabinets (Mitchell 1956) for 3–4 weeks prior to harvest. The plants received 12 hours of light per day at an intensity of approximately 2,700 foot candles, and with a spectral energy distribution close to that of sunlight. Relative humidity was between 70% and 80%. Temperatures were held constant at the levels indicated in Tables 7 and 8.

The plants used in these studies were chosen to include common representative grasses and legumes, i.e. perennial ryegrass (*Lolium perenne*), short-rotation ryegrass (*Lolium perenne* × *L. multiflorum*), paspalum (*Paspalum dilatatum*), cocksfoot (*Dactylis glomerata*), browntop (*Agrostis tenuis*), Yorkshire fog (*Holcus lanatus*), white clover (*Trifolium repens*), subterranean clover (*Trifolium subterraneum*), and lotus major (*Lotus uliginosus*).

When the growth period was completed the plants were harvested, plants in each experiment being cut at the same time, (9–10 a.m. for material in Tables 1 and 2 and 11 a.m. for material in Table 5), and the material immediately freeze dried. After drying, the samples were ground and stored in paraffin-sealed jars until analysed.

Alcohol Extraction

Dried powdered plant material (4 g) was blended for 3 minutes in a Waring Blendor with about 200 ml 80% alcohol (V/V). After centrifugation the residue was extracted a further three times in the same way and the supernatants combined and evaporated to dryness in a weighed flask in a rotary vacuum drier. Drying was completed in a vacuum desiccator containing calcium chloride, constant weight usually being attained within 48 hours. After weighing, the dried extract was gently shaken with 10–20 ml water, the supernatant being filtered off. Three further extractions with water sufficed to remove all the water-soluble material. This solution was evaporated, dried, and weighed in the same way as the previous extract, its weight indicating the amount of material soluble both in water and alcohol. The difference between the two weights corresponded to the material soluble in alcohol and insoluble in water.

Water Extraction

The residue from the alcohol extraction was blended for three minutes in the Waring Blendor with about 200 ml water containing 0.5 ml acetic acid. After centrifugation the residue was extracted again in the same way. The combined supernatants were dried in a rotary vacuum evaporator and then in a vacuum desiccator over calcium chloride. This constituted the water-soluble, alcohol-insoluble fraction. In the first experiment, the insoluble residue was dried and weighed, but in the two succeeding experiments the weight of the alcohol-insoluble, water-insoluble fraction was obtained by difference.

In some cases amino-acids and total amino nitrogen were measured in the alcohol-insoluble, water-soluble fraction. In order to set free the amino-acids, the dried extract was hydrolysed by refluxing overnight with 10 ml 1:1 HCl. The acid was removed by evaporation to dryness on a steam bath and residue taken up in 80% alcohol, the alcohol removed, and the extract made to a convenient volume with water.

Analytical Methods

Soluble nitrogen was determined in the water and alcohol extracts by the micro-Kjeldahl method using copper as catalyst, followed by distillation into boric acid and titration with 0.01N HCl. Before digestion, nitrate was reduced with iron.

Total nitrogen was measured by the macro-Kjeldahl or micro-Kjeldahl method, depending on the amount of material available.

Insoluble nitrogen was obtained by difference.

Amino nitrogen was measured by the Van Slyke nitrous acid method and also by summation of the figures obtained for individual amino-acids.

Nitrate nitrogen. A suitable aliquot (e.g. 1 ml) of the alcohol extract was placed on a column of the anion-exchange resin Amberlite IR 4B in the hydroxide form, and the column washed thoroughly with water. The nitrate was then eluted with 50 ml N NaOH, followed by water to bring the eluate to 100 ml. Five ml or other suitable aliquot was then taken for estimation of nitrate by the xylenol method (Jones and Underdown 1953).

Amino-acids were estimated by quantitative paper chromatography using the method of Wellington (1952).

Total sugars were estimated by the ferricyanide method after hydrolysis of the extract with 0.15N H_2SO_4 and measured as glucose (Fujita and Iwatake 1932).

Ash was determined on a 1 ml aliquot by weighing the residue after drying and ignition at 450°.

Organic acids. A 1 ml aliquot of the alcohol extract was passed through a column of the anion-exchange resin Amberlite IRA 400 (carbonate form). After the column had been thoroughly washed with water, elution was performed with N ammonium carbonate. Excess ammonium carbonate was removed by boiling and the ammonium salts converted to free acids by passage of the solution through a column of the cation-exchange resin Amberlite IR 112 (hydrogen form). The eluate, together with washings, was evaporated to dryness, taken up in water, re-evaporated four times (to remove volatile acids), and titrated with 0.02N NaOH using phenolphthalein as indicator. Organic acids were calculated as malic acid.

Fructosans. 3 ml water extract, together with 27 ml water and 0.075 g oxalic acid in a 50 ml volumetric flask was heated at 80° for 1 hour. After cooling and neutralisation with barium hydroxide, the hydrolysate was made to 50 ml. The liberated reducing sugar was measured by the ferricyanide method as for total sugars.

Water-soluble carbohydrate was measured colorimetrically by the anthrone method (Morris 1948).

RESULTS

In the first experiment, three grasses (perennial ryegrass, short-rotation ryegrass, and paspalum) and three legumes (white clover, subterranean clover, and lotus major) were grown in soil in pots. On the alcohol extracts the following estimations were made: total sugars, total soluble nitrogen, amino nitrogen, nitrate nitrogen, and individual amino-acids. The water extracts were hydrolysed and used for estimations of total soluble nitrogen, total amino nitrogen, and individual amino-acids.

Results for this experiment are set out in Tables 1, 2, 3, and 4. Insufficient material was available for the estimation of total nitrogen in the white-clover OL sample. In Tables 3 and 4 "others" represents the sum of the amino nitrogen from serine, threonine, glycine, valine, leucine, isoleucine, and often some unknown amino-acids. Individual amino-acids in the water extracts are not shown as the total quantity was small and variations did not show a discernible pattern.

Tables 5 and 6 set out the results of the second experiment, a trial on white clover using three levels of light. The same analyses were performed as for the previous experiment.

TABLE 1. Group Analyses of Grasses Grown Under Different Conditions of Temperature and Light Intensity

	Short-rotation Ryegrass				Perennial Ryegrass				Paspalum			
	GL	GS	OL	OS	GL	GS	OL	OS	GL	GS	OL	OS
Solids %—												
Sol. alc. Insol. water	10.6	10.3	8.4	10.3	8.3	8.7	5.7	11.7	7.0	11.3	8.3	10.3
Sol. alc. Sol. water	25.7	23.0	31.3	22.7	25.0	19.3	27.7	23.3	22.0	17.0	24.7	19.0
Insol. alc. Sol. water	6.0	5.0	9.0	12.3	12.0	7.3	12.7	7.3	6.3	5.7	6.7	6.3
Insol. alc. Insol. water	58.0	56.0	45.3	58.0	52.0	58.0	50.0	53.8	65.3	65.0	60.3	62.7
Total	100.3	94.3	94.0	103.3	97.3	93.3	96.1	96.1	100.6	99.0	100.0	98.3
Alc. extract—												
Total sugars %	4.94	2.07	5.16	5.13	6.01	1.34	6.93	4.49	6.18	2.19	9.84	3.72
Sol. N mg%	1120	982	161	254	143	1025	158	246	119	883	164	518
NH_2—N mg%	224	215	83	123	104	127	68	105	61	89	89	96
NO_3—N mg%	775	792	9	12	5	816	0	43	36	715	9	380
Water extract—												
Sol. N mg%	53	28	57	99	54	41	45	67	41	55	51	75
NH_2—N mg%	35	22	26	38	17	20	20	31	20	26	25	39
Total sol. nitrogen mg%	1173	1010	218	353	197	1066	203	313	160	938	215	593
Total nitrogen %	5.39	4.28	2.82	3.23	3.06	3.96	2.55	3.42	1.62	2.42	1.22	1.61
Insoluble nitrogen %	4.22	3.27	2.60	2.88	2.86	2.80	2.35	3.11	1.46	1.48	1.01	1.02
Total sol. N as % of total N	22	24	8	11	6	27	8	9	10	39	18	37

GL = Glasshouse Light GS — Glasshouse Shade OL — Outside Light OS = Outside Shade

TABLE 2. GROUP ANALYSES OF LEGUMES GROWN UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND LIGHT INTENSITY

	White Clover				Subterranean Clover				Lotus Major			
	GL	GS	OL	OS	GL	GS	OL	OS	GL	GS	OL	OS
Solids %—												
Sol. alc. Insol. water	6.3	7.3	7.7	9.3	8.7	7.7	8.3	7.3	10.4	8.7	10.6	10.4
Sol. alc. Sol. water	28.0	29.7	26.0	30.0	21.7	20.0	27.7	24.7	25.3	27.3	28.7	26.3
Insol. alc. Sol. water	12.0	8.0	11.0	7.7	4.7	4.7	18.0	10.0	2.7	8.7	10.3	9.3
Insol. alc. Insol. water	51.7	52.0	55.0	51.3	56.7	57.3	52.3	54.0	52.3	55.0	48.7	53.0
Total	98.0	97.0	99.7	98.3	96.8	94.7	96.3	96.0	95.7	99.7	98.3	99.0
Alc. extract—												
Total sugars %	4.71	2.96	6.21	7.22	4.74	1.66	7.61	3.90	4.25	4.18	9.50	4.75
Sol. N. mg%	242	1050	431	336	237	590	189	274	304	1050	406	630
NH_2—N mg%	72	172	144	83	118	153	153	168	194	214	194	278
NO_3—N mg%	31	496	17	51	12	156	0	7	8	633	10	153
Water extract—												
Sol. N mg%	73	73	103	56	66	109	96	95	44	30	54	48
NH_2 mg%	41	35	48	33	22	45	42	57	19	13	32	32
Total sol. nitrogen mg %	315	1123	534	392	303	699	285	369	344	1080	460	678
Total nitrogen %	3.40	3.91		3.46	3.65	3.93	3.40	3.43	2.72	3.95	3.27	3.31
Insoluble nitrogen %	3.09	2.79		3.07	3.35	3.23	3.11	3.06	2.37	2.87	2.81	2.63
Total sol. N as % of total N	9	29		11	8	18	8	11	13	27	14	20

GL = Glasshouse Light GS = Glasshouse Shade OL = Outside Light OS = Outside Shade

TABLE 3. INDIVIDUAL FREE AMINO-ACIDS OF GRASSES GROWN UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND LIGHT INTENSITY

	Short-rotation Ryegrass				Perennial Ryegrass				Paspalum			
	GL	GS	OL	OS	GL	GS	OL	OS	GL	GS	OL	OS
Aspartic acid	26	24	9	13	13	13	7	18	7	10	2	13
Asparagine	39	51	2	14	9	24	tr	9	0	14	tr	6
Glutamic acid	12	20	6	6	12	12	7	5	4	10	9	17
Glutamine	25	16	6	13	14	13	3	6	tr	2	1	2
Alanine	27	19	7	9	12	14	10	10	6	17	12	16
γ-aminobutyric acid	35	13	11	23	18	19	14	33	3	4	2	6
Others	23	24	4	11	12	28	10	15	2	10	5	11
Total	187	167	45	89	90	123	51	96	22	67	31	71

Results expressed as mg NH_2—N per 100 g dry matter.
tr = trace.

TABLE 4. INDIVIDUAL FREE AMINO-ACIDS OF LEGUMES GROWN UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND LIGHT INTENSITY

	White Clover				Subterranean Clover				Lotus Major			
	GL	GS	OL	OS	GL	GS	OL	OS	GL	GS	OL	OS
Aspartic acid	7	16	12	12	6	9	11	19	10	9	13	15
Asparagine	12	43	41	11	14	53	19	28	18	27	51	48
Glutamic acid	9	18	18	9	10	10	8	9	6	5	13	8
Glutamine	4	2	5	2	4	2	2	2	2	1	2	2
Alanine	7	11	14	6	5	5	5	4	9	5	8	9
γ-aminobutyric acid	5	7	11	5	8	5	2	7	6	7	11	8
Others	7	19	17	7	5	13	5	5	2	4	6	19
Total	51	116	118	52	52	97	52	74	53	58	104	109

Results expressed as mg NH_2—N per 100 g dry matter.

Results of the third trial conducted on plants grown in cabinets are shown in Tables 7 and 8, which show figures for three representative species out of the nine used in the experiment. In addition to paspalum, short-rotation ryegrass, and subterranean clover (included in the tables), the other species investigated were: browntop, Yorkshire fog, cocksfoot, perennial ryegrass, white clover, and lotus major. The range of analyses were somewhat modified—amino-acids in the water extracts and Van Slyke amino nitrogen in both alcohol and water extracts were omitted, and ash, organic acids, fructosans (on grass samples), and total water-soluble carbohydrates (on clover samples) included. Some gaps in the paspalum results were due to insufficiency of material.

TABLE 5. GROUP ANALYSES OF WHITE CLOVER GROWN UNDER DIFFERENT LIGHTING CONDITIONS

	Full Light	One Layer Scrim	Two Layers Scrim
Solids %—			
Sol. alc. insol. water	7.0	7.4	6.5
Sol. alc. sol. water	22.7	22.9	22.5
Insol. alc. sol. water	10.7	10.3	8.7
Insol. alc. insol. water	55.7	54.3	55.7
Total	96.1	94.9	93.4
Alcohol extract—			
Total sugars %	6.68	5.94	5.37
Soluble N mg%	880	1030	1160
NH_2—N mg%	205	196	157
NO_3—N mg%	463	692	927
Water extract—			
Soluble N mg%	86	98	73
NH_2—N mg%	46	53	40
Total soluble nitrogen mg%	966	1128	1233
Total nitrogen %	4.65	4.58	4.24
Insoluble nitrogen %	3.68	3.45	3.01
Total sol. N as % of Total N	21	25	29

TABLE 6. FREE AMINO-ACIDS OF WHITE CLOVER GROWN UNDER DIFFERENT LIGHTING CONDITIONS

	Full Light	One Layer Scrim	Two Layers Scrim
Aspartic acid	5	6	3
Asparagine	81	62	39
Glutamic acid	7	7	2
Glutamine	5	4	3
Alanine	38	35	33
γ-aminobutyric acid	17	17	17
Others	14	11	8
Total	167	142	105

DISCUSSION

The first experiment (Tables 1, 2, 3, and 4) showed that the different levels of light and temperature were accompanied by very great differences in some of the constituents. Sugar content followed the expected pattern, being highest with full light and low temperature and lowest with shade and high temperature. Soluble nitrogen showed marked changes, these being due mainly to the changes in nitrate, which was almost always highest (and usually extremely high) in plants

TABLE 7. Group Analyses of Plants Grown in Controlled Environment Cabinets

		Paspalum						Short-rotation Ryegrass					Subterranean Clover			
		45°F	55°F	65°F	75°F	85°F	95°F	45°F	55°F	65°F	75°F	85°F	45°F	55°F	65°F	75°F
Dry matter %—																
Sol. alc. insol. water				17.3	6.1	9.4	5.6	6.3	7.1	5.3	7.9	8.7	12.2	9.7	6.4	7.4
Sol. alc. sol. water				21.9	21.7	20.3	23.3	31.2	28.1	27.4	24.6	24.8	27.1	28.9	24.1	23.8
Insol. alc. sol. water					8.3	4.9	5.0	7.5	6.2	5.7	6.6	6.5	9.2	5.8	8.8	7.9
Insol. alc. insol. water					63.9	65.4	66.1	55.0	58.6	61.6	60.9	60.0	51.5	55.6	60.7	60.9
Total nitrogen	%			3.45	4.19	3.27	3.34	4.28	5.23	4.34	3.94	4.69	3.54	4.04	4.42	4.67
Nitrate-N	mg%	623	547	480	610	600		468	580	980	1140	1110	84	200	228	450
Alcohol-sol. N	mg%	625	580	715	728	710	1070	925	847	1130	1230	1203	338	326	447	754
Water ext. N	mg%	140	110	74	117	82	62	62	62	28	69	62	41	47	63	92
Total soluble N	mg%	765	690	789	845	792	1132	987	909	1158	1299	1265	379	373	510	846
TSN as % of TN				23	20	24	34	23	17	27	33	27	11	9	12	18
Alc. sol. N × 6.25	%	3.9	3.6	4.5	4.6	4.4	6.7	5.8	5.3	7.1	7.1	6.9	2.1	2.0	2.8	4.7
Sugars	%	6.7	11.0	3.9	4.4	6.1	3.9	13.0	9.1	3.9	3.3	5.8	7.3	3.2	3.0	2.1
Ash (alc. ext.)	%			7.9	6.3	7.0	3.1	5.8	6.8	4.7	6.9	6.0	5.6	6.2	6.2	6.7
Organic acids	%	6.8	2.9	2.2	0.6	1.4	1.3	3.2	2.4	1.9	4.5	1.4	3.4	3.6	2.9	2.8
Total identified solids alcohol ext.	%			18.5	15.9	18.9	15.0	27.8	23.6	17.6	21.8	20.1	18.4	15.0	14.9	14.3
Water ext. N × 6.25	%	0.9	0.7	0.5	0.7	0.5	0.4	0.4	0.4	0.2	0.4	0.4	0.3	0.3	0.4	0.6
Water ext. ash	%			1.4	2.0	0.8	1.7	1.0	1.3	3.2	2.3	2.3	0.9	1.9	3.3	3.3
Fructosans	%	0.9	0.8	1.1	0.5	0.2	0.2	2.6	1.2	0.7	0.2	0.3	—	—	—	—
Water ext. total carbohydrate	%	—	—	—	—	—	—	—	—	—	—	—	0.8	0.8	0.6	0.7
Total identified solids water ext.	%			3.0	3.2	1.5	2.3	4.0	2.9	4.1	2.9	3.0	2.0	3.0	4.3	4.6

TABLE 8. FREE AMINO-ACIDS IN PLANTS GROWN IN CONTROLLED ENVIRONMENT CABINETS AT DIFFERENT TEMPERATURES

	Paspalum						Short-rotation Ryegrass					Subterranean Clover			
	45°F	55°F	65°F	75°F	85°F	95°F	45°F	55°F	65°F	75°F	85°F	45°F	55°F	65°F	75°F
Aspartic acid	2	4	6	3	7	33	12	18	26	44	13	2	4	2	2
Asparagine	50	23	28	5	6	31	24	12	13	25	21	32	29	32	33
Glutamic acid	4	8	8	3	12	36	18	26	27	68	21	1	3	2	2
Glutamine	11	7	41	4	11	14	98	66	52	27	23	0	0	0	0
Alanine	46	30	55	25	9	21	47	25	25	18	66	24	31	31	34
γ-aminobutyric acid	44	46	57	43	30	4	37	34	32	29	45	9	24	24	27
Others	59	19	31	8	15	18	20	22	29	22	32	22	14	8	11
Total	216	137	226	91	90	157	255	203	204	233	221	90	105	99	109

Results expressed as mg NH_2—N per 100 g dry matter.

grown at high temperature in the shade. Negligible amounts of nitrate were found in plants grown at the lower temperature in full light. Differences in amino nitrogen were not so striking but it was usually found that the shade-grown plants were higher in amino nitrogen than the corresponding plants grown in full light. Differences in individual amino-acids show little apparent regularity in the different species except in the case of the amides glutamine and asparagine. Asparagine was usually highest in the plants grown at high temperature in the shade and lowest in those grown in full light. Differences in glutamine content were less, the highest contents being obtained in the two glasshouse series.

The amount of insoluble nitrogen did not vary greatly for the different treatments, whereas the total nitrogen showed much greater variation. Although it is a common convention to designate as "crude protein" the amount of total nitrogen multiplied by 6.25, and, indeed, this convention is followed in this paper in Table 5, it is widely recognised that this practice can be misleading. The extent and variation of this discrepancy between total and insoluble nitrogen can perhaps be most clearly seen in the last row of Table 1 showing the total soluble nitrogen as a percentage of the total nitrogen.

When the sum of the amino nitrogen and nitrate nitrogen was subtracted from the total nitrogen, there was usually a deficit, sometimes quite large, but little significant regularity could be detected in the differences between treatments. The deficit was usually larger in clovers than in grasses.

The partition of the dry matter on the basis of solubility in alcohol and in water, revealed unaccountable and often large variations between the various fractions, but no regular pattern of differences between treatments is seen. Some differences between species appear, notably the larger amount of insoluble material in paspalum, and of material soluble in both alcohol and water in white clover.

Some anomalies appear in the results, especially with white clover and short-rotation ryegrass. In the case of white clover, sugars appear higher in the OS than in the OL samples. Many of the nitrogenous constituents in these two samples also show a reverse tendency when compared with the other clovers. The amount of nitrate in the GL sample of short-rotation ryegrass is very high, its value almost equalling that of the GS sample. Because of these anomalies, it was decided to repeat the trial the following summer (1955). Detailed figures for this experiment are not quoted as they followed, in general, those for the first experiment. Variations in nitrate content, although still considerable, were less extreme than in the previous trial, as nitrate was usually fairly high even in the light-grown samples. This was probably due to the plants being grown in vermiculite supplied with nutrient solution containing nitrate, whereas previously soil was used. In every case except one, however, the nitrate increased with decreasing light, the exception being, significantly, the samples of short-rotation ryegrass grown in the glasshouse. Sugars, too, showed less variation than before although the trend of the results was, in general, in the same direction, i.e. increasing with increasing light and decreasing temperature. There were some exceptions, however, these including the glasshouse samples of lotus and paspalum and the outside samples of white clover. It will be recalled that in the first experiment recorded here the outside-grown white clover also showed more sugar in the shade than in the light.

The experiment with white clover using three levels of light intensity (Tables 5 and 6), revealed a good correlation between light intensity and the content of various constituents, the results agreeing in general with those obtained for the GL and GS samples in the first set. Nitrate levels were, however, much higher, owing to the different culture medium. Clover plants grown in the field usually contain much less, if any, nitrate, as the usual practice is to grow clover in association with grass which preferentially takes up the available nitrate. Amino nitrogen dropped with decreasing light, whereas in the first experiment it increased. Of the individual amino-acids, asparagine occurred in the greatest quantity and showed the greatest change, the amount decreasing markedly with decreasing light.

For the final experiment reported here (see Tables 7 and 8) in which the plants were grown in cabinets under controlled temperature conditions, the range of analyses was extended in order to obtain a more complete characterisation of the two soluble fractions. In the case of the grasses, the dry matter of the alcohol extracts was largely accounted for by the analyses performed, whereas with the clovers a considerable amount of undetermined material is indicated. Recoveries for the water extracts were usually far from complete.

Fructosan was low in comparison with some results from overseas (Waite and Boyd 1953). It should be noted, however, that the purpose of the work here reported was to characterise the water-extract constituents rather than to achieve complete extraction of fructosan.

In many cases the difference between the amounts of total soluble nitrogen and nitrate nitrogen was less than the amount of amino nitrogen. Discrepancies of this kind have been repeatedly observed, particularly with grasses high in nitrate, and are believed to be due to the loss of some nitrogenous material during the Kjeldahl digestion (Butler *et al.* pers. comm.).

Nitrate tended to increase with increasing temperature, whereas amino nitrogen tended to decrease, this latter figure being influenced mainly by the contents of asparagine and glutamine. Differences in the culture medium are reflected in much higher amounts of amino nitrogen being found than in the first experiment, particularly in the case of short-rotation ryegrass (compare Table 8 with Tables 3 and 4). The highest sugar values were usually found in the low-temperature samples but occasionally samples grown at the higher temperatures showed more sugar than corresponding samples grown at lower temperatures.

ACKNOWLEDGEMENTS

Thanks are due to Miss J. Wheeler and Miss J. Strawbridge for technical assistance.

REFERENCES

FUJITA, A.; IWATAKE, D. 1932: *Biochem. Z. 242:* 43-60.

JOHNS, A. T. 1955: *N.Z. J. Sci. Tech. A37:* 301-11.

JONES, G. B.; UNDERDOWN, R. E. 1953: *Anal. Chem. 25:* 806-8.

MITCHELL, K. J. 1956: *N.Z. J. Sci. Tech. A38:* 203-16.

MORRIS, O. L. 1948: *Science 107:* 254.

WAITE, R.; BOYD, J. 1953: *J. Sci. Food Agric. 4:* 197-204.

WELLINGTON, E. F. 1952: *Canad. J. Chem. 30:* 581-91.

MOLYBDENUM DEFICIENCY OF THE HOP

By H. O. Askew, R. J. Monk, and Joyce Watson,
Cawthron Institute, Nelson.

(*Received for publication, 12 March 1958*)

Summary

A necrosis of hop leaves, eventually leading to a white speckling of the lamina, has been identified as a result of a deficiency of available soil molybdenum.

Applications of sodium molybdate at 16 oz per acre were very effective in overcoming the ailment. Ground limestone at 1800 lb per acre was also very beneficial, but not quite to the same degree as sodium molybdate.

Leaves showing the symptoms contain only about 0.02 p.p.m. molybdenum in ~~the~~ dry matter of the whole lamina. Levels of other minor elements—copper, zinc and manganese—do not appear to be related to the incidence of leaf symptoms.

Improvement in health of leaves and growth of plants is correlated positively with increased molybdenum content of the leaves whether that increase is brought about by use of sodium molybdate or of ground limestone. Molybdenum content of the cones is similarly increased.

There is a suggestion in the data that size of cones may be increased, and that the sugars contents of cones may be reduced, following use of sodium molybdate.

INTRODUCTION

In recent years molybdenum has come into prominence as an essential nutrient element for plants and a number of field and horticultural crops are now known to respond favourably to applications of molybdenum compounds after exhibiting various leaf and growth abnormalities. It was very interesting to find therefore in a sample of hop leaves showing a form of breakdown of the lamina leading to white speckling of the surface that the molybdenum content of such leaves was very low. Thus in the 1953-54 season, when attention was drawn to this abnormality, the molybdenum content of the dry matter of the lamina was only 0.025 p.p.m. Other minor elements were present in these leaves as follows: zinc, 57 p.p.m.; copper, 15 p.p.m.; manganese, 340 p.p.m. Apparently healthy leaves from other parts of the same garden gave the following figures on analysis: molybdenum, 0.04 p.p.m.: zinc, 42 p.p.m.: copper, 14 p.p.m.: manganese, 209 p.p.m. These samples were taken from the main bine at a height of four to five feet from the ground. Many samples of leaves have been analysed from a number of field trials in various parts of the Waimea County of Nelson, practically the only part of New Zealand where hops are grown, over several years, with the following ranges of values for the amounts of the various minor elements in the leaf laminae: molybdenum, 0.08–2.1 p.p.m.; zinc, 23–53 p.p.m.; copper, 11–21 p.p.m.; manganese, 58–520 p.p.m. Thus the data obtained for the abnormal leaves lie within the ranges of values for zinc, copper and manganese

N.Z. J. agric. Res. 1: 553–568.

for normal material, but the molybdenum figure is much below normal. Since in the samples from other parts of the district there was no suggestion of a similar abnormality it was considered possible that the affected leaves were suffering from a deficiency of molybdenum. The present paper describes experimental work to examine this possibility.

A preliminary account of the first two seasons' work has already been published (Watson and Askew, 1956), and notes on later work are to be found in the Annual Reports of the Cawthron Institute (Askew and Watson, 1956; Askew, Monk, and Watson, 1957).

Occurrence of Symptoms

Although the same symptoms on leaves have been seen in other hop gardens the most severe occurrence was in a garden at Neudorf, in the Moutere district of Nelson, in the Californian variety, which is the main variety grown in the Waimea County. Young shoots coming through the ground following pruning of the hills carry chlorotic leaves which after expansion show a light-brown spotting of the interveinal areas; these brown spots change to an almost white colour later (Fig. 1). Edges of the leaves are upcurled and the leaf becomes very harsh and brittle (Fig. 2). Many of the leaves may grow to an almost normal size on the upper parts of the bine, but severely affected bottom leaves remain small, and later fall from the plants. In extreme cases affected leaves may occur practically to the top of the plants, the symptoms following up the bine as it climbs. When the symptoms are

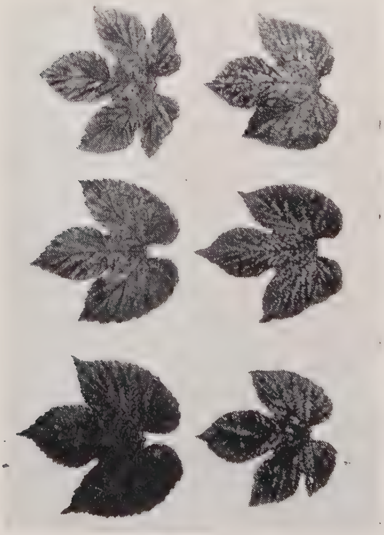


Fig. 1

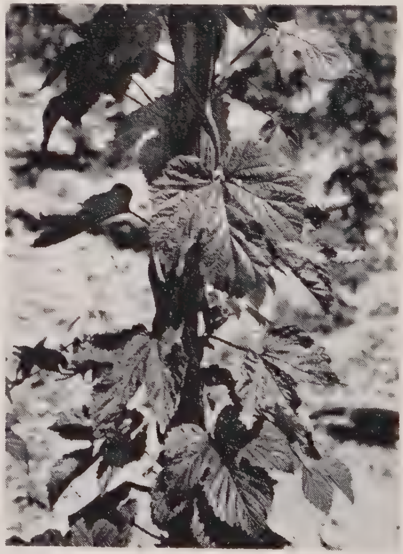


Fig. 2

Fig. 1.—Healthy leaf bottom left; other leaves showing increasing degrees of severity of symptoms. (Californian variety), December 1955.

Fig. 2.—Speckling and upcurling of leaves due to molybdenum deficiency (Californian variety), December 1955.

mild, only the lower leaves may be affected, so that above four to five feet above the ground level all leaves may be apparently healthy. Affected plants flower and carry apparently normal-shaped hops carrying the usual lupulin. Severely affected plants may carry few cones but even badly affected plants may have a fair crop of cones.

EXPERIMENTAL

The hop garden is situated at Neudorf on the Motupiko soil type (silt loam phase) of Nelson, with a pH value of 5.4 to 5.7. The plants (Californian variety) were grown on poles until the 1957-58 season when wirework was erected. When the experiments began in 1954-55 the garden was in its fourth harvest season. Previously to being planted the land had been in pasture for sixteen years, receiving one ton of ground limestone per acre when sown and annual dressings thereafter at 5–6 cwt per acre. A normal commercial hop fertiliser was used in the garden each season.

In the original series of experimental plots set out on 21 October 1954, six hills (3 × 2) were used with guard rows on all sides. The ten plots were allocated at random in the block of plants without any previous knowledge of the distribution of affected hills: it was only known that the area selected had been badly affected in the previous season. All the hills received the usual commercial fertiliser and cultural treatments of the grower. On this day too the grower broadcast borax throughout the garden at the rate of 50 lb per acre. The experimental treatments, with the number of replications given by the bracketed figures, were as follows:

Untreated (3): no treatment.

Sodium molybdate (3): 0.25 g per hill, applied in aqueous solution (equivalent to about 8 oz molybdate per acre).

Ground limestone (2): 2 lb per hill broadcast over the area (equivalent to approximately 1800 lb per acre).

Molybdate plus ground limestone (2): combination of the two previous treatments.

The dressings of molybdate and limestone were repeated on 25 October 1955 at the same rates as in the previous season; since then no further applications have been made.

New plots (western and eastern series) on which the hills received sodium molybdate at 16 oz per acre were established on 25 October 1955. These have not received any further molybdate.

On 21 October 1954, when the first applications were made, the plants were carrying short shoots, some of which were chlorotic; of the thirteen hills so affected four were on untreated plots, six on molybdate, two on limestone, and one on the combined treatment plots. This chlorosis has been found in the absence of suitable treatment to be a fore-runner of the leaf symptoms which later affect the leaves. On 16 December all the plants that received only sodium molybdate were healthy: of the twelve treated with ground limestone seven were healthy, one was slightly, three were appreciably, and one was severely, affected by the leaf condition already described; molybdate plus limestone provided only one slightly affected plant out of the twelve treated, while from the eighteen plants of the untreated plots seven were apparently healthy, four were appreciably, six were badly, and one was very badly, affected. By 2 February 1955 the plants of the untreated plots had deteriorated still further, hardly any plants now being healthy; some slight regression occurred on the limestone and limestone plus molybdate plots, but no deterioration of the plants receiving only molybdate was noticed. On this date many of the plants in the guard rows were badly affected with the leaf symptoms, some to the extent that they were almost dead. At harvest on 3 March 1955 all the plants except one receiving only molybdate bore healthy dark-green leaves and a good crop of cones; the exception showed fairly bad symptoms on the leaves. Ground limestone in conjunction with molybdate gave well grown plants carrying a good crop. Limestone when used alone was not so satisfactory especially on one plot where the plants were in poor condition and carried smaller hops than normal. Plants on the untreated plots were mostly showing severe leaf symptoms, although they had a fair crop of cones. Use of sodium molybdate was therefore very beneficial to the plants, allowing production of generally healthy leaves and a good crop of hops. The condition of the untreated (Fig. 3) and the molybdate treated plots (Fig. 4) just previous to harvest is shown in the photographs.

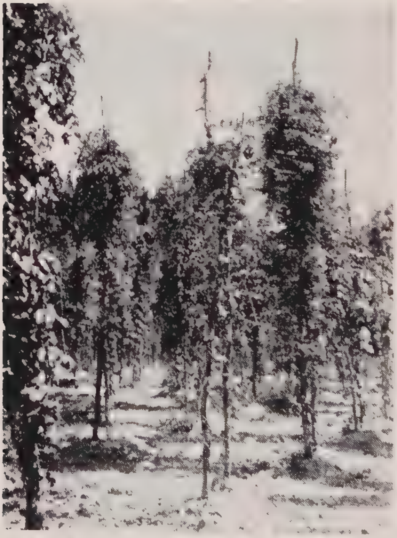


Fig. 3

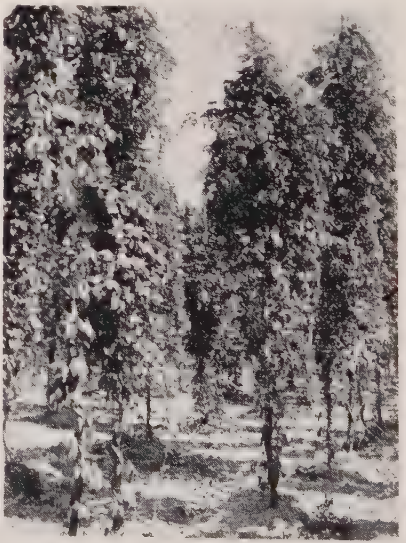


Fig. 4

Fig. 3.—Untreated plot, Californian variety, about three weeks before harvest, on 7 February 1955.

Fig. 4.—Plot receiving sodium molybdate at 8 oz per acre in previous spring; photographed, about three weeks before harvest, on 7 February 1955.

In the 1955-56 season the first inspection of the plots was not made until 9 December 1955. At this time all the plants receiving molybdate only were well grown with fresh green leaves; molybdate plus limestone were not so good, some slight symptoms being visible. Ground limestone was fairly satisfactory although the plants were not in such good condition as those receiving molybdate. Nearly every one of the untreated plants showed severe symptoms. A further inspection was made on 20 December when the following conditions were found (figures in brackets refer to the number of plants in the plots):

Untreated (18): 1 healthy, 4 showing slight symptoms, 13 had severe symptoms.

Molybdate only (18): 15 healthy, 2 with trace of symptoms, 1 dead from other causes.

Molybdate plus limestone (12): 8 healthy, 4 with trace of symptoms.

Limestone only (12): 5 healthy, 5 with trace of symptoms, 2 badly affected.

Later in the season further inspections were made on 23 January and 21 February 1956, the latter being a few days before harvest. On the former date the plants were well in burr; no definite difference in stage of development between treated and untreated plants was noted. Essentially the same condition of the plants was found on these two dates as on 20 December. All molybdate-treated plants were fresh and green with a good crop of hops: with limestone only most of the plants were dark green with a good crop. In spite of the poorer condition of most of the plants on the untreated plots there was a reasonable crop; on one plot, however, where affected leaves were present right to the top of the plant, the cones were smaller than normal. Plants in guard rows around the plots were often affected with the typical leaf symptoms, and with leaves smaller, and of a lighter green colour, than normal.

The first inspection for the 1956-57 season of the experimental plants was made on 26 October 1956. Pruning of the hills had been completed but there were very few shoots showing as yet; some shoots were however about nine inches long. Nearly all the plants with shoots appeared to be healthy, but a few had yellowed shoots. On 10 December some of the bines had climbed only about two feet up the poles, but others had barely started climbing. Affected plants were seen, especially on the untreated plots and in the guard rows. The molybdate-treated plots had the better-grown plants. Counts for grading of the plants were made on 21 December 1956 and on 1 March 1957. The results as percentages are shown below, the symbols being: H, healthy; T, trace of symptoms; M, medium; and S, severe, expression of symptoms.

These figures show that in the earlier part of the season there were no noteworthy differences between the condition of the plants on the various treatments, but that at harvest the molybdate-treated plots were appreciably, and the molybdate plus limestone and the limestone

Treatment	21 Dec., 56				1 Mar., 57			
	H	T	M	S	H	T	M	S
Untreated	80	17	3	0	61	22	0	17
Molybdate	80	10	10	0	100	0	0	0
Limestone	75	25	0	0	92	8	0	0
Molybdate plus limestone	73	18	9	0	73	17	0	0

plots were somewhat, healthier than the untreated ones. The improved condition of all treated plots must have been due to the loss of formerly affected leaves at the base of the plants.

In the 1957-58 season examinations were made on 5 November 1957, and on 20 January and 26 February 1958. On the former date all the plants appeared to be free from leaf symptoms, although some were yellowish or relatively weak. On the last two dates grading of the plants gave the following results, expressed as percentages of the plants receiving the stated treatments:

Treatment	20 Jan., 58				26 Feb., 58			
	H	T	M	S	H	T	M	S
Untreated	83	0	11	6	76	6	6	12
Molybdate	100	0	0	0	100	0	0	0
Limestone	100	0	0	0	100	0	0	0
Molybdate plus limestone	100	0	0	0	100	0	0	0
Guard rows	83	8	8	1	91	0	8	1

The effects of the treatments in this season are somewhat more favourable than in the previous one, all treated plots being healthy, but the untreated ones show some symptoms. It is interesting to note that weak or off-coloured plants were more frequent on the untreated and limestone plots; these were recorded as "healthy", being free from leaf symptoms, but they were not as vigorous or of such good colour as fully healthy plants would be. At the time of the last recording there was a good crop of hops on all treated plots but on untreated ones, although most plants carried quite good crops, some of the plants had a below-average crop.

Further sets of plots were established on 25 October 1955, one on either side of the original plots. In one set (western) two replications, each containing twelve hills (4 × 3), were left untreated or received sodium molybdate at 16 oz per acre; the plots were suitably protected by guard rows. On this area to the west of the original plots somewhat badly affected plants were present in the previous season. On 20

December 1955 all of the plants in this western series, except three very slightly affected ones, which had received molybdate, were healthy, but on untreated plots only fourteen were healthy or slightly affected, while four were appreciably, and six were seriously, affected with the leaf spotting. By 23 January 1956 only ten plants on the untreated plots were healthy compared with twenty-two on the treated plots. Later again in the season, on 21 February, all plants except a few with a trace of symptoms were carrying leaves of deep-green colour and large crops of well grown hops: on the untreated plots, although many plants showed the leaf symptoms, quite good crops of hops were being carried. In the guard rows surrounding the plots many plants were in poor condition, with severely affected leaves and little top growth.

In the following season, on 10 December 1956, the molybdenum-treated plants were healthy, but were not so advanced in growth as the corresponding treated plants on the original plots started a year earlier. Further examinations were made on 21 December 1956 and 1 March 1957, when the plants were graded, using the same symbols as previously. The percentages of plants in the several categories are given below, together with the data for plants in the guard rows:

	21 Dec., 56				1 Mar., 57			
	H	T	M	S	H	T	M	S
Molybdate plots	82	14	4	0	100	0	0	0
Untreated plots	80	12	8	0	88	8	4	0
Guard rows	79	17	4	0	86	7	3	4

These figures show that on 21 December there was little difference in the condition of the plants, whether treated or not, but that by 1 March the molybdate-treated plants were better than those on the untreated plots or in the guard rows. It would appear that affected leaves on some of the plants that received molybdate must have fallen off between December and March. Moreover the incidence of the ailment was appreciably less in the 1956-57 season than in the previous one.

Inspections of the plots have been made in the 1957-58 season on the same dates as those for the original plots. On 5 November 1957 practically all the plants, treated or untreated, appeared to be healthy, but no actual counts were made. But on 20 January and 26 February 1958, the latter date just previous to harvest, the plants were graded. On the former date ninety-two per cent of the untreated plants were graded as healthy; no plants showed any severe incidence of symptoms. In the guard rows ninety-five per cent were healthy and five per cent showed severe symptoms. On the molybdate-treated plots all plants were healthy. At the end of February all plants on both untreated and treated plots, and in guard rows, were apparently healthy, but more off-colour and less vigorous plants were present in untreated plots and the guard rows than on the treated plots. Molybdate treatment appeared to produce a heavier crop of cones than where it was omitted.

The second new set of plots established on 25 October 1955 was to the east of the original plots on an area not so severely affected as the locations of the other plots. Four replications, using plots of twenty plants (4 × 5), of untreated and molybdate-treated plots at 16 oz sodium molybdate per acre, were provided. By 9 December the favourable effect of the molybdate was very pronounced although not a hundred per cent effective. The results of further inspections of the plants on 20 December 1955 and on 23 January 1956, using the same symbols as before and giving these as percentages, are set out below:

	20 Dec., 55				23 Jan., 56			
	H	T	M	S	H	T	M	S
Molybdate plots	90	10	0	0	82	18	0	0
Untreated plots	64	16	18	2	53	26	16	5
Guard rows	59	17	17	7				

These figures demonstrate clearly the beneficial effect of the applications of sodium molybdate even in a season when the incidence of the ailment was not as marked as in 1954-55. The data for the guard rows check satisfactorily with those of the untreated plots. At a later date, 21 February, just before harvest, the plants carried good strong leafage and a good crop of hops, with a trace only of leaf symptoms on the molybdate plots, but with a number of severely affected plants on the untreated plots; on these poor plants the crop was lighter.

No further application of molybdate was made in the 1956-57 season. Grading of the plants was carried out on 21 December 1956 and on 1 March 1957. On the former date only a trace of leaf symptoms was seen on both treated and untreated plots, and on the latter there was very slightly more of the symptoms on the untreated plants; there was no real difference in the condition of the plants which could be ascribed to treatment. This last season was apparently one in which the symptoms were much reduced, as has already been seen when discussing the other plots.

In the 1957-58 season there was no difference between the treated and untreated plants, all being essentially healthy.

Effect of Sodium Molybdate on Size and Dry-matter Content of Hop Cones

It has seemed at times that the plants on plots receiving molybdate and limestone were carrying larger hops than those on untreated plants. Samples of hops have therefore been obtained from the three sets of plots. Individual samples have been taken on the ten original plots, taking one hundred cones per plant (six hundred cones per plot); on the western supplementary plots twelve hills were used to obtain nine hundred cones per sample, and on the large eastern plots two plots were combined from which twelve hills were chosen to give nine hundred cones per sample. Green weights and dry weights, from which the percentages of dry matter were calculated, have been determined. The results for samples obtained on 21 February 1956 and 27 February 1957 are given in Tables 1 and 2 respectively, the green and dry weights being expressed in grammes per thousand cones.

TABLE 1. AVERAGE GREEN AND DRY WEIGHTS (g/1000 CONES) AND PERCENTAGES OF DRY MATTER OF HOP CONES, 1956.

Series	Treatment	Green Weight (g)	Dry Weight (g)	Dry Matter (%)
Original	Untreated (3)	741	120.2	16.3
,,	Molybdate (3)	722	123.1	17.0
,,	Molybdate plus limestone (2)	765	125.1	16.3
,,	Limestone (2)	667	114.1	17.1
,,	General average (10)	715	120.8	16.6
Western	Untreated (1)	787	135.5	17.2
,,	Molybdate (1)	849	141.1	16.6
Eastern	Untreated (2)	—	122.0	—
,,	Molybdate (2)	—	131.5	—

TABLE 2. AVERAGE GREEN AND DRY WEIGHTS (g/1000 CONES) AND PERCENTAGES OF DRY MATTER OF HOP CONES, 1957.

Series	Treatment	Green Weight (g)	Dry Weight (g)	Dry Matter (%)
Original	Untreated (3)	644	131.9	20.5
,,	Molybdate (3)	697	128.4	18.5
,,	Molybdate plus limestone (1)*	653	112.2	17.2
,,	Limestone (2)	640	112.6	17.6
,,	General average (9)	662	124.2	18.8
Western	Untreated (1)	737	119.4	16.2
,,	Molybdate (1)	642	121.8	19.0
Eastern	Untreated (2)	679	122.2	18.0
,,	Molybdate (2)	782	149.3	19.0

* Data for one sample were accidentally lost.

In both Table 1 and Table 2 the figures in brackets show the number of plot samples that were taken to obtain the mean figures given for each treatment. In the 1956 season there were appreciable variations between replicates from the original (smallest) plots which probably are sufficient to nullify statistically the indications of effect from treatment in Table 1. Thus the individual data for the percentage of dry matter in the samples from the ten plots of the original series lead to a figure of 0.25% for the standard error of the mean value. The figures of the right hand column of Table 1 show that any differences due to treatment on dry-matter content are doubtful statistically. Similarly the standard error of the mean value of the dry weights is 2.6; on this basis only the limestone plot may be significantly lower in yield than the others. In Table 2 this treatment again has given a low figure for the yield of dry matter. It is interesting to note that use of molybdate gave increased yields of dry matter per thousand cones in all three series of plots in 1956, but only on the western and eastern series in 1957. The effect of molybdate on percentage of dry matter has been inconsistent in both seasons.

Therefore on the basis of the present data it is not possible to say that use of molybdate has had any effect on size of cone, as judged by amount of dry matter, or on the percentage of dry matter in the cone. The dry-matter data from the larger plots (eastern and western) with their better conditions for sampling are very suggestive of benefit from molybdate.

CHEMICAL DATA

Soil Analyses

Samples of soil were obtained before the experimental plots were laid out, when it was found that the pH values varied from 5.3 to 5.6 in different parts of the garden. Further samples from the individual plots of the original series were taken on 26 March 1956, about eighteen months after the experiments began, during which time a total of 16 oz sodium molybdate and 3600 lb ground limestone per acre had been used on the plots specified for these treatments. Composite samples, at 0–6 in. and 6–12 in. depths, with eight or nine 2 in. diameter cores per treatment, were taken on both the original and the eastern and western series of plots; pH values and molybdenum and manganese soluble in hot concentrated hydrochloric acid were determined. The data obtained are set out in Table 3, where the molybdenum and manganese contents are expressed in parts per million of dry soil.

The data of Table 3 show that the soil of this hop garden was distinctly acid in most parts; there is variation however as shown by the range 5.35 to 6.0 in the pH values of the soil from unlimed plots. The second depth of soil has varied over practically the same range. Plots treated with limestone have the highest pH values, both in the topsoil and in the second depth of sampling, but considering that 3600 lb per acre of limestone has been used on this silt loam the increase in pH value has not been large. The uniformity between the pH values of the samples from the two depths is explained by the mixing of the soil by cultivation.

Molybdenum content of this soil is low, averaging only about 0.15 p.p.m. of acid-soluble molybdenum in the top twelve inches. Use of

TABLE 3. pH VALUES AND ACID-SOLUBLE MOLYBDENUM AND MANGANESE IN SOILS FROM EXPERIMENTAL PLOTS

Plot Series	Treatment	Depth of Sampling (inches)	pH	Mo	Mn
Original	Untreated	0– 6	5.7	0.20	977
,,	Untreated	6–12	5.5	0.12	1049
,,	Limestone	0– 6	6.25	0.10	1038
,,	Limestone	6–12	6.2	0.20	1307
,,	Molybdate	0– 6	6.0	0.33	862
,,	Molybdate	6–12	6.0	0.13	711
,,	Molybdate plus limestone	0– 6	6.1	0.20	1155
,,	Molybdate plus limestone	6–12	6.0	0.27	1241
Western	Untreated	0– 6	5.4	0.10	770
,,	Untreated	6–12	5.55	0.13	770
,,	Molybdate	0– 6	5.35	0.40	878
,,	Molybdate	6–12	5.6	0.10	821
Eastern	Untreated	0– 6	5.7	0.13	1049
,,	Untreated	6–12	5.75	0.07	1036
,,	Molybdate	0– 6	5.8	0.80	1049
,,	Molybdate	6–12	5.4	0.13	1034

sodium molybdate at the rates specified above has increased the acid-soluble molybdenum by 50% on the original series of plots, and has more than doubled it on the other two series: but by reason of the smaller amount of cultivation since application on the western and eastern plots the bulk of the applied molybdate still remains in the top six inches of soil. The field observations detailed above have shown that the added molybdate has been available to the plants; it will be shown in the next section of this paper that determinations of the molybdenum contents of leaves and cones from these plots confirm the uptake of this element.

Manganese content of the soil is high, ranging from 711 to 1241 p.p.m. On the plots of low pH value high availability of manganese, leading to the appearance of large amounts in leaves and cones, is to be expected. In view too of possible relationships between uptake of molybdenum and manganese the soil figures are useful.

Analyses of Leaves and Cones

Leaves. The first samples of leaves from this garden were taken on 21st January 1954 from the main bine of the plants at a height of four to five feet. Two of them showed chlorosis or speckling while the third was rated healthy. Chemical data for these samples are given below, the figures being parts per million in the dry matter for specified elements:

These figures suggested that lack of molybdenum might be the cause of the symptoms on the leaves.

Condition	Zinc	Copper	Manganese	Molybdenum
Chlorotic	61	17.0	331	0.02
Speckled	54	12.6	353	0.03
Healthy	42	13.9	209	0.04

In the following season the first experimental plots were set out and, when samples of leaves were taken on 16 December 1954 from the plots, samples were also taken of badly curled and speckled and of healthy leaves from other parts of the garden. The chemical data (p.p.m.) from these samples were as follows:

Condition	Zinc	Copper	Manganese	Molybdenum
Speckled	59	10.1	257	0.02
Healthy	58	12.7	287	0.03

Again the affected leaves are lower in molybdenum than the healthy ones; variations between the other constituents are not considered significant.

Leaf samples from the original plots were taken on 16 December 1954, that is, about two months after the treatments were applied, and on 2 February 1955. Chemical data, using average figures from similarly treated plots, are given in Table 4, expressed as parts per million of element in the dry matter:

The data show that on both sampling dates the amounts of zinc, copper, and manganese in the leaves are not related to the treatments applied, but that the molybdenum content has been raised where sodium molybdate had been applied. Reference to an earlier section

TABLE 4. MINOR ELEMENTS IN HOP LEAVES (p.p.m. of D.M.)

Treatment	Zinc		Copper		Manganese		Molybdenum	
	16/12/54	2/2/55	16/12/54	2/2/55	16/12/54	2/2/55	16/12/54	2/2/55
Untreated	55	40	11.2	—	229	229	0.02	0.04
Molybdate	54	36	9.8	—	240	303	0.07	0.10
Limestone	55	38	10.9	—	211	231	0.04	0.05
Molybdate plus limestone	51	38	10.7	—	200	248	0.07	0.08

of this paper shows that increased level of molybdenum in the leaves is closely correlated positively with health of the plants and with incidence of leaf symptoms. It is of interest to note that leaves from a part of the garden which has not been affected by leaf symptoms taken on the same date in February as those from the experimental plots showed a molybdenum content of 0.08 p.p.m., practically identical with the figure obtained for leaves from plants receiving molybdate treatment. It is interesting to note too that the older the leaves the more molybdenum and manganese, but the less zinc, they contained.

In the 1955-56 season samples were taken from the original plots, which were re-treated in this season, and from the new western and eastern plots started in this season with applications of sodium molybdate equivalent to 16 oz per acre, on 23 January for leaves and 21 February 1956 for cones. Nitrogen, as well as manganese and molybdenum, was determined on these samples. Only the mean figures for the different treatments are given in Table 5.

TABLE 5. NITROGEN (%) AND MANGANESE AND MOLYBDENUM (p.p.m.) OF THE DRY MATTER OF HOP LEAVES AND CONES, 1956 HARVEST

Lines	Treatment	Leaves			Cones		
		N	Mn	Mo	N	Mn	Mo
Original	Untreated	2.84	413	0.12	2.79	113	0.52
	Limestone	2.99	299	0.23	2.68	78	0.61
	Molybdate	2.93	254	1.03	2.52	92	1.39
	Molybdate plus limestone	2.79	226	0.66	2.69	73	1.44
Western	Untreated	2.62	314	0.10	2.55	109	0.79
	Molybdate	2.83	371	1.08	2.71	76	1.59
Eastern	Untreated	—	327	0.23	—	84	0.56
	Molybdate	—	325	0.33	—	98	1.31

The data of Table 5 show that the nitrogen and manganese contents of both leaves and cones have not been affected consistently by the treatments applied, although the reduction in manganese content of the cones from use of limestone may be real, but that the molybdenum content has been increased by the use of ground limestone and especially following use of molybdate. Nitrogen contents of leaves are somewhat higher, and manganese contents are much higher, than those of cones. The increase in molybdenum content of leaves from 0.1–0.2 p.p.m. in samples from untreated plots to 0.3–1.1 p.p.m. where molybdate was used is very marked; even the raising of the figure to 0.23 p.p.m. from 0.12 p.p.m. by use of ground limestone is appreciable. Similarly the treatments applied have markedly increased the molybdenum contents of the cones. Another notable point is the high molybdenum content of cones compared with the corresponding leaves from the untreated plots. In each case too cones from the variously treated plots are richer in molybdenum than the corresponding leaves.

Reference to the earlier section of this paper where the effects of the treatments on health of the plants was discussed will show how closely improved health and freedom from or reduction in severity of leaf symptoms was positively correlated with increased molybdenum content of the leaves and cones, although no physical effects of treatment on the cones has been noted.

Further chemical data are given in Table 6, where samples of leaves and cones taken on 27 February 1957 are reported upon. Nitrogen, manganese, and molybdenum contents have been determined.

TABLE 6. NITROGEN (%) AND MANGANESE AND MOLYBDENUM (p.p.m.) OF THE DRY MATTER OF HOP LEAVES AND CONES, 1957 HARVEST.

Series	Treatment	Leaves			Cones		
		N	Mn	Mo	N	Mn	Mo
Original	Untreated	2.63	258	0.05	2.43	113	0.05
	Limestone	2.28	200	0.04	2.65	96	0.05
	Molybdate	2.77	267	0.08	2.69	131	0.12
	Molybdate plus limestone	2.45	171	0.14	2.45*	90*	0.16*
Western	Untreated	2.73	291	0.04	2.84	106	0.05
	Molybdate	2.72	208	0.04	2.63	123	0.10
Eastern	Untreated	2.64	249	0.05	2.56	112	0.05
	Molybdate	2.52	252	0.12	2.52	101	0.15

* One sample only.

The data of Table 6 follow those of Table 5 in the irregular effect of the treatments on nitrogen content, and to some extent on manganese content, with the exception that use of limestone has reduced the manganese present in both leaves and cones. Nitrogen contents of both leaves and cones are generally lower in 1957 than in 1956; this is true also for the manganese content of the leaves. In 1957 the manganese in the cones tended to be higher than in the previous season.

Molybdenum contents of both leaves and cones were much lower in 1957 and tended to be comparable with the 1955 figures rather than those of 1956. The effect of the molybdate dressings is quite marked in raising the molybdenum content of both leaves and cones, except in the case of the leaves from the western set of plots. Limestone in this season has not affected the molybdenum content of either leaves or cones. There is in this season too no evidence of the cones being so much richer than the leaves in molybdenum as was found in 1956. It may be noted however that cones from healthy gardens have been found to be richer in molybdenum than the corresponding leaves (unpublished data). Although the molybdenum contents of the leaves in 1957 appear to be low, yet they are well above the limit of about 0.02 p.p.m. which seems to be associated with the appearance of the symptoms ascribed to a lack of molybdenum. This is confirmed too by the field observations detailed in a previous section, where it was noted that there was no difference in the plants in this season which could be ascribed to treatment; in fact all were essentially healthy.

Sugar Content of Cones

In view of the known variation in the amounts of sugars in hop cones it was of interest to see whether the treatments used in the present experiments affected the amounts of glucose, fructose, and sucrose in the cones. Samples taken on 27 February 1957, just before harvesting began, have been analysed. Glucose is that sugar or sugars reacting with hypoiodite and fructose is the difference between this figure and the figure for total reducing sugars determined by the Somogyi copper reagent. Sucrose is calculated from the increase in total reducing sugars following mild hydrolysis. The data obtained are set out in Table 7. Glucose content of the samples from the control plots ranges from 1.05% to 1.30%, and has in all cases been reduced by use of molybdate by about 0.3% from the corresponding untreated figure. In the two comparisons possible, use of limestone has raised the glucose content. At the present time owing to insufficient experimental evidence it is not possible to give reasons for these reductions from the levels of the controls. Fructose contents have also been reduced where molybdate has been used, actually not being found, that is the level is below 0.1% in some samples. Sucrose has either not been found or is present in very small amount, not much beyond the probable accuracy of the methods employed.

TABLE 7. Glucose, Fructose, and Sucrose Contents of Hop Cones (% of D.M.), 1957 Season.

Series	Treatment	Glucose	Fructose	Sucrose
Original	Untreated	1.2	0.5	0.0
	Molybdate	1.0	0.1	0.0
	Limestone	1.35	0.25	0.0
	Molybdate plus limestone	1.3*	0.4*	0.0*
Western	Untreated	1.3	0.2	0.1
	Molybdate	0.9	0.0	0.0
Eastern	Untreated	1.05	0.15	0.1
	Molybdate	0.75	0.0	0.1

* One sample only.

Obviously further information is required on the effect of molybdate, limestone, and fertilisers on the sugar content (and other constituents) of the hop cone.

DISCUSSION

The data presented above leave no doubt about the conclusion that molybdate applications have been very effective in overcoming the symptoms on the leaves. Use of ground limestone has likewise been beneficial. From the known fact that dressings of limestone make molybdenum more available to plants it can be concluded that the effectiveness of the limestone is linked with increase in available molybdenum, more particularly as chemical analysis showed that the molybdenum content of leaves and cones was raised.

Use of limestone has tended to decrease the intake of manganese by the plants, but the applications of sodium molybdate have not consistently affected the amount of manganese in the leaves and cones. It was however of interest to see whether molybdenum and manganese would show any antagonistic or other effects in the hop plant.

So far as is known, the incidence of the leaf symptoms, which can now be fairly claimed to be due to a deficiency of available molydenum, is not widespread in Nelson hop gardens. These symptoms have been seen elsewhere than the garden at Neudorf where the above investigation was carried out. Although the Motupiko soil type is known to be molybdenum-deficient to other crops, and therefore unlimed hop gardens on it may suffer from sub-optimal supplies of available molybdenum, there is no information at present which would lead to a recommendation for the general use of sodium molybdate in those gardens.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. A. J. Waters for most of the nitrogen figures used in this paper, and for assistance in the collection of the samples. They are also appreciative of the action of Mr. H. A. D. Clouston in permitting this investigation to be carried out in his garden.

REFERENCES

ASKEW, H. O.; WATSON, JOYCE 1956: Cawthron Inst. annu. Rep. 1955-56, p. 45.

ASKEW, H. O.; MONK, R. J.; WATSON, JOYCE 1957: *Ibid. 1956-57*, p. 50.

WATSON, J.; ASKEW, H. O. 1956: *Nature, Lond. 178:* 1302.

STUDIES OF GRAZING OF CROP FORAGES BY SHEEP

I. THE EFFECT OF GRAZING ON COMPOSITION, DIGESTIBILITY, AND NUTRITIVE VALUE OF RAPE

By A. F. Greenall, Crop Research Division, Department of Scientific and Industrial Research, Lincoln*

(*Received for publication, 6 January 1958*)

Summary

The composition and digestibility of ungrazed rape and of rape selected for similarity to that chosen by grazing wethers were determined. The composition of the rape consumed by these wethers was deduced from the content and digestibility of proximate constituents of the fæces. The digestibility of dry matter declined with delayed sowing and grazing. The digestive capacities of three wethers, for rape, were almost identical; a fourth was 2% lower. The digestibility of selected rape was only 0.4% higher than that of ungrazed rape and the difference was not significant. The composition of selected rape was intermediate between that of ungrazed and grazed rape. However, the latter was markedly lower in ether extract and crude protein and higher in crude fibre. Hand selection of rape similar to that chosen by the grazing wethers was thus only partly successful. The digestibility of leaf was little more than 2% higher than that of stem plus petiole, a smaller difference than was expected. Despite real differences in chemical composition, the nutritive value of grazed rape was only slightly lower than that of ungrazed rape. This illustrates the important effect of organic matter digestibility on food value.

Although selective grazing clearly occurred, the effect on food value was negligible.

Introduction

In comparing varieties within species, the yields of forage crops are usually measured by cutting off the herbage at ground level, despite the evidence, mainly from pasture research, that animal techniques are desirable for critical comparisons (Ahlgreen 1947; Sears 1951). In Europe and North America, where it is customary to cut and feed out forage crops, yields obtained from cutting at ground level provide a more accurate prediction of animal production than they would in New Zealand where *in situ* grazing is usual. The practice of measuring yield by cutting at ground level, though useful for preliminary screening of varieties, requires the assumption that production of animals grazing these is proportionate to yield, an assumption that may be invalid and misleading. Usually, the yields of different species are measured by similar techniques and compared on the common basis of units of starch equivalent (S.E.) by the use of standard S.E. values. However, Lancaster and Adams (1943) have shown large differences between the S.E. of rape grown in New Zealand and in the United Kingdom, where standard S.E. values in current use originated. Occasionally digestibility determinations are carried out concurrently with measurements of yield, which are then expressed as units of S.E. per acre (Allison and Thurston 1952). Though this is probably the

---

* Now Chief Soil Conservator, Otago Catchment Board, Dunedin.

N.Z. J. agric. Res. 1: 569–581.

best non-animal technique for critical comparisons, it does not take into account the habits of grazing animals.

For pasture research Ahlgreen (1947) and others have emphasised the need for studies on the effects of trampling, soiling, and selective grazing on utilisation. This also applies to crop forages as it is clear from observation of normal grazing that not only do animals graze selectively, trample, and soil forage, but they also reject stubble residues in amounts varying according to species, stage of development, weather, intensity of grazing, and grazing management. Furthermore wastage in grazing occurs owing to dropping, dirtying, and destruction of herbage, and there is a wastage in digestion and metabolism, shown by the excretion of dung and urine. The latter is voided indiscriminately, but much falls on leaves. Doak (1952) has shown that urea, which in urine comprises about 70% of the total nitrogen, may be absorbed by leaves with a consequent increase in forage nitrogen. Where this absorption does not raise herbage nitrogen above about 8.5% protein equivalent, an increase in digestibility, and consequently in food value, is likely (Head 1953; Mitchell *et al.* 1940; Gallup *et al.* 1954).

As stubble is mainly stems and basal portions of petioles, which differ from leaves chemically and morphologically, leaf/stem ratios are sometimes determined. This is meaningless for animal production unless the food values of leaf and of stem plus petiole are measured in digestibility experiments. It follows from the above considerations that the nutritive value of the portion of the rape crop consumed by a grazing animal could be quite different from that of an ungrazed rape crop cut at ground level. For critical comparisons a better understanding is required of the effect of grazing on the composition, digestibility, and feeding value of rape, as well as of other crop forages.

METHODS

Four adjacent quarter-acre blocks of Broad-leaf Essex II rape were drilled at the Crop Research Division, Lincoln, on the following dates in 1951: block I 18 October, block II 1 November, block III 15 November and block IV 3 December. The blocks were on the same soil type, had been through the same cropping sequence, and received the same husbandry treatment except for time of sowing. Establishment was satisfactory and weeds were absent. When ripe for grazing, each block was sampled for yield and divided into two unequal parts, of equal dry-matter yield per unit area. One part was grazed with sixteen two-tooth half-bred wethers, while the other part was left ungrazed (Fig. 1). Four similar wethers were trained in digestibility pens, and divided into two pairs, one of which was pre-fed for five days on rape from the ungrazed area, the other pair being fed selected rape from the grazing area. The height of stems that would be left as stubble under normal grazing management was estimated and the ungrazed rape was cut at this height (Fig. 2). Selected rape was chosen from the grazing area on the basis of its similarity to that being selected by the sixteen grazing wethers. The daily ration for each wether in the digestibility experiment was 9 lb green weight, about three-fifths of which was fed at 5 p.m. and the balance at 8 a.m. The ration was

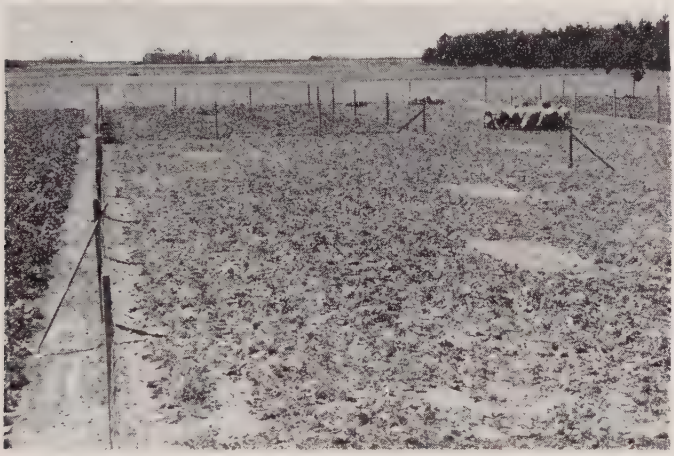


Fig. 1.—Rape block grazed by wethers.

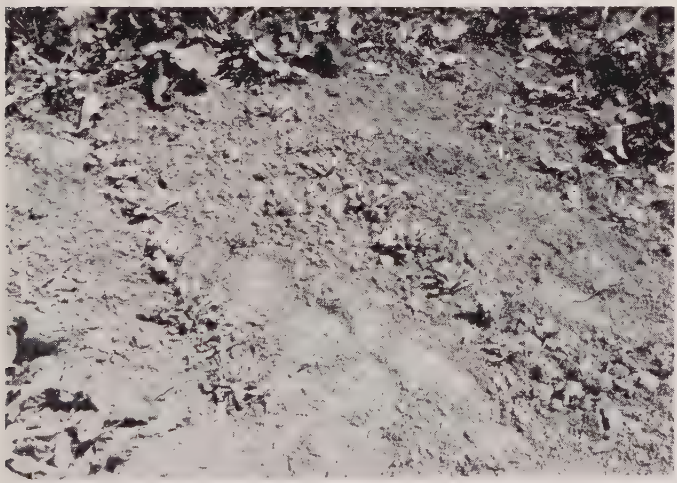


Fig. 2.—Ungrazed rape cut at height to which it would normally be grazed.

slightly less than would be consumed by feeding to appetite, in order to reduce selection by the wethers to a minimum. The parts of the rape plants eaten on successive days by the sixteen grazing wethers were carefully observed, and, as just stated, similar feed was chosen for the pair of digestibility wethers eating selected rape. The nature of the feed selected ranged from leaf on the first day to stem and petiole on the eighth day. The pair of digestibility wethers eating ungrazed rape was fed a ration which was similar throughout the eight-day collection period. The daily intake of dry matter and its chemical composition were determined from duplicate subsamples. Aliquots of feed, of feed refused, and of fæces, were taken at the end of each eight-day period and analysed by the standard A.O.A.C. method. As soon as the appearance of the grazing area, or the behaviour of the sheep, indicated that intake was falling unduly, the wethers were moved on to the next block. To eliminate some of the bias due to sheep variability, the pair that had been eating ungrazed rape in blocks I and II was fed the selected rape from blocks III and IV. The four blocks were grazed over a total period of 32 days.

What is considered to be a good estimate of the apparent digestibility of leaf and of stem of grazed rape was made as follows. It was observed that feed selected on the first day was mainly leaf and on the eighth day was mainly stem plus petiole, and that the ratio of leaf to stem decreased regularly. Consequently the ratio of leaf to stem on any one day of the eight-day period could be determined, as could the leaf-to-stem ratio on two four-day sub-periods. These ratios for the first and the second sub-periods were equated to the apparent digestibility coefficient of the first and the second sub-periods respectively, and the two equations solved. It is not unusual to divide digestibility periods into sub-periods (Raymond *et al.* 1954) though a sub-period of longer than four days would have been desirable to reduce the error due to "end effect" (Blaxter *et al.* 1956).

The following example is given for sheep 3 fed selected rape from block II:

First sub-period, fed 22/28 leaf (L) and 6/28 stem (S), digestibility 87.4%,

second sub-period fed 6/28 L and 22/28 S, digestibility 86.0%

$$22/28\ L + 6/28\ S = 87.4\%$$

$$6/28\ L + 22/28\ S = 86.0\%$$

Solving, digestibility of leaf = 87.9% and of stem = 85.5%.

The eight-day digestibility periods of ungrazed rape were also divided into two four-day sub-periods in the same manner. With ungrazed rape, however, the same feed was given on each day so that the difference between the digestibility coefficients of these two sub-periods provided a measure of the error involved in the method used.

RESULTS

At least two of the stall-fed wethers lost weight during the four periods. However, as shown in Table 1 these losses were small, indicating that intake was at about the maintenance level.

TABLE 1. LIVE WEIGHTS (lb) OF THE FOUR WETHERS USED FOR DIGESTIBILITY DETERMINATIONS.

Sheep No.	Block I	II	III	IV
	Weight On	Weight Off	Weight On	Weight Off
No. 1	93	88.5	88.5	89.4
2	85	82.5	82.5	84.9
3	90	90.4	90.4	89.8
4	87	86.5	86.5	83.4

TABLE 2 (a): APPARENT DIGESTIBILITY COEFFICIENTS OF DRY MATTER OF RAPE.

Sheep No.	Block I				II				III				IV				Sheep Mean
	Ungrazed Sub-period		Selected Sub-period		Ungrazed Sub-period		Selected Sub-period		Ungrazed Sub-period		Selected Sub-period		Ungrazed Sub-period		Selected Sub-period		
	1st	2nd	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	86.5	86.2			86.1	86.1					87.2	85.9			85.4	83.5	85.8
2	86.9	86.2			85.0	84.0					85.9	86.5			84.3	82.3	85.1
3			88.0	85.7			87.9	85.5	86.4	85.0			83.5	85.4			85.9
4			86.9	83.8			86.0	81.4	83.7	82.5			83.5	82.5			83.8
Block mean	86.2				85.2				85.3				83.7				
Feed mean	Ungrazed; sub-period 1 = 85.2, 2 = 84.7 Ungrazed = 84.9								Selected; sub-period 1 = 86.4, 2 = 84.3 Selected = 85.3								

The apparent digestibility coefficients of dry matter and an analysis of variance of these data are recorded in Tables 2 (a) and 2 (b) respectively. From the nature of the experiment it was not possible to separate out interactions between the block, sheep, and feed effects as their interpretation would be ambiguous. They are included in the 22 degrees of freedom for error. Each pair of sheep on a block on one feed provides one degree of freedom for "true error" and the mean square for these eight degrees of freedom is 0.5847. The mean square for the remainder of the error degrees of freedom is 1.0080 which is not significantly greater.

TABLE 2 (b): ANALYSIS OF VARIANCE OF DATA IN TABLE 2 (a).

Variation Due to	d f		M.S.	
Blocks	3		8.40**	
Linear		1		21.24***
Other		2		1.98
Sheep	3		7.90**	
Type of Feed	1		1.40	
Sub-periods (Ungrazed)	1		0.86	
(Selected)	1		18.06***	
Error	22		0.8541	

Coefficient of Variation = 1.1%.

*** Significant at the 0.1% level of probability.
** Significant at the 1% level of probability.

Effect of Time of Sowing and Grazing on the Digestibility of Rape

The differences in digestibility of rape between blocks were significant at the 1% probability level. Blocks received identical treatment apart from being sown and grazed in order from block I to block IV. It is clear from Table 2 that there was a reduction in digestibility of rape with later sowings and grazings. While the differences were not large, the linear relationship between delayed sowings and grazings and reduced digestibility of rape was significant at the 0.1% probability level. As the weather was normal during the period over which the rape was grown and grazed, the evidence of a small but definite decline of digestibility with delayed sowing and grazing suggests a seasonal effect either on the digestive capacity of sheep or on the digestibility of rape.

Variability in the Capacities of Sheep for Digesting Rape

The differences between the digestive capacities of the four wethers were significant at the 1% level of probability. This was due mainly to the slightly lower digestive capacity of sheep 4. The digestive capacities of the remaining three wethers were surprisingly similar in view of differences recorded by several other investigators. The lower capacity of sheep 4 is thought to be characteristic of this wether rather than due to temporary nervous or digestive upsets, of which there were no outward signs.

Digestibility of Dry Matter of Leaf and of Stem

Leaf/stem ratios are of value only if the nutritive differences between leaf and stem are known. Rape leaf, at the lamb-fattening stage, contains a higher concentration of crude protein (C.P) and ether extract (E. Ex.) and a lower concentration of crude fibre (C.F.) than stem and petiole. Little is known concerning the digestibility of leaf and of stem of forages, though differences between these are frequently assumed to be large. For instance Hardison *et al.* (1955) have shown appreciable differences of digestibility between the upper and the lower parts of lucerne at the hay stage.

The apparent digestibility coefficients of leaf and of stem of selected rape are shown in Table 2 (a). The apparent digestibility of sub-period 1 of selected rape is estimated digestibility of leaf whereas that of sub-period 2 is that of stem plus petiole. With ungrazed rape there was close agreement between the digestibilities of sub-periods whereas with selected rape the digestibility of feed of the second sub-period (stem) was, with one exception, lower than that of the first (leaf). The accuracy of these determinations is dependent on the validity of the assumption of a regular change in the proportion of leaf and stem consumed and on a 1:1 leaf to stem plus petiole ratio. It is considered that these assumptions fit the observations so well that good estimates of the digestibility of leaf and of stem were obtained. Though the mean difference between the digestibilities was only about two per cent, it is shown in the analysis of variance part of Table 2 that the digestibility of rape leaf was significantly higher than that of stem, at the 0.1% level of probability.

Digestibility of Ungrazed and of Selected Rape

The apparent digestibility coefficient of dry matter of selected rape was slightly higher than that of ungrazed rape but the difference of 0.4% was not significant and was highly significantly less than 1.4%.

It was possible to undertake Henneberg analyses of only blocks II and III. The apparent digestibility coefficients of proximate constituents and of organic matter are shown in Table 3.

Selected rape had a significantly higher digestibility of C.F. and significantly lower digestibility of E.Ex. than ungrazed rape. The feed differences were, however, very small except for E.Ex. As rape contains so little ether extract its low digestibility is unlikely to cause appreciable differences in feeding value between selected and ungrazed rape.

Composition of Ungrazed, Selected, and Grazed Rape

That sheep graze pasture selectively may be readily observed and is generally accepted. Selective grazing not only of species but also of different parts of the herbage of one species is thought to occur. If this is so and also in view of the different compositions of leaf and of

TABLE 3. APPARENT DIGESTIBILITY COEFFICIENTS OF PROXIMATE CONSTITUENTS OF UNGRAZED AND SELECTED RAPE.

Replicate	Crude Protein		Crude Fibre		Ether Extract		Nitrogen-free Extract		Organic Matter	
	Ungrazed	Selected	Ungrazed	Selected	Ungrazed	Selected	Ungrazed	Selected	Ungrazed	Selected
1	71.9	77.4	83.7	86.7	34.4	20.1	94.6	96.1	89.0	91.0
2	73.2	72.6	85.0	86.4	20.0	0.0	95.0	94.7	89.0	88.8
3	79.9	72.1	88.5	90.1	35.4	25.9	93.9	94.3	89.4	89.9
4	75.2	75.4	87.2	89.8	23.7	20.6	93.3	94.1	87.3	89.7
Mean	75.0	74.4	86.1	88.2	28.4	16.7	94.2	94.8	88.7	89.8

Analyses of Variance

Variation due to:	d.f.	MS	F	MS	F	MS	F	MS	F	MS	F
Feed	1		N.S.		30.8*		10.68*		2.57		3.63
Replicates	3		N.S.		27.4*		6.35		3.93		1.24
Error	3	14.95		0.30		25.74		0.28		0.76	
C.V.		5.0%		0.6%		23.0%		0.6%		0.9%	

* Significant at the 5% probability level.

stem plus petiole, it follows that the composition of preferred forage will differ from that of the whole plant. It also follows that fæces of animals grazing pasturage would differ from those of animals fed clipped or mown forage, as found by Hardison *et al.* (1951) and Hobbs *et al.* (1945). The first quantitative measures of selective grazing of lucerne were recorded by Hardison *et al.* (1954), and of sudan grass and lucerne by Bath *et al.* (1956), who used an esophageal fistula technique. In both studies there was a higher percentage of crude protein and of ash in the grazed than in the clipped feed.

The comparative smallness of leaves of many pasture species, the general use of a mixture of species in pastures, the intermingling of aerial parts of plants as well as the recognised structural and chemical differences between different portions of the same plant have made impossible the manual selection of pasture forage identical with that selected by grazing animals. However, selection should be possible in pure stands of rape, which has large leaves, petioles, and stems. Despite these advantages it will be seen in Table 4 that selection has not been wholly successful. In Table 4 are presented the chemical composition and nutritive value of rape eaten by the grazing wethers and of ungrazed rape.

TABLE 4. CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF RAPE EATEN BY WETHERS GRAZING *in situ*, AND OF UNGRAZED AND OF SELECTED RAPE

Block	Feed	Composition — % Organic Matter				Nutritive Value		
		Crude Protein %	Crude Fibre %	Ether Extract %	Nitrogen-free Extract %	T.D.N.	S.E.	Nutritive Ratio
I	Grazing wethers	7.70	21.3	0.9	70.1			
II	Grazing wethers	9.3	15.2	1.1	74.4	91.4	82.9	12.1
	Selected	11.2	13.1	2.3	73.4	90.3	83.4	9.7
	Ungrazed	10.9	12.7	3.4	73.0	90.1	83.1	10.4
III	Grazing wethers	9.8	16.9	1.6	71.7	90.8	81.2	11.6
	Selected	9.9	13.7	2.8	73.6	90.4	83.2	11.3
	Ungrazed	12.0	13.5	3.6	70.9	88.9	82.5	8.7
IV	Grazing wethers	10.9	9.8	1.5	77.8			

The composition of the feed selected by the grazing wethers was reconstructed from fæcal composition and the appropriate coefficients of digestibility by a method similar to that employed by Hardison *et al.* (1954). However, Raymond *et al.* (1954) and others suggest that calculation from digestibility coefficients multiplies errors. It is clear from Table 4 that there were differences in composition between grazed and selected rape, and so there would probably be differences in digestibility. It is unlikely however that this would be of an order greater than the 0.4% difference in digestibility between selected and ungrazed rape shown in Table 2 (a). The higher C.F. and lower C.P. and E.Ex. content of rape eaten by the grazing wethers suggest that its food value is likely to be slightly lower than that of selected rape.

Nutritive Value

The nutritive value of a feed may be expressed as total digestible nutrients (T.D.N.) or as starch equivalents (S.E.) with the nutritive ratio. Values for T.D.N. of ungrazed, selected, and grazed rape were determined by the method described by Tyler (1950) and are shown in Table 4. S.E. and nutritive ratio were calculated by the method of Woodman (1957) except that in place of Y values a correction was made for fibre according to standard practice with high-fibre forages. There was very little difference between the three feeds in either T.D.N. or S.E., though the nutritive ratio of grazed rape was unexpectedly wide. This mean ratio of about 12 is considered to be not very suitable for fattening, whereas that of about 9.5 for ungrazed rape is satisfactory. Despite the empirical nature of the correction for calorific value and percentage of C.F. involved in determinations of S.E., these are to be preferred to T.D.N. as a measure of food value. It must be concluded then that grazed rape was slightly poorer in food value than ungrazed rape. With feeds of normal content of C.F. and of E.Ex. it is evident that the level of organic matter digestion largely determines nutritive value. This is implied by the method of Lofgreen (1953) of calculating T.D.N. from the digestibility of organic matter. As the organic matter digestibilities of ungrazed and selected rape were similar, so were their feed values. This is mainly due to the similarity of calorific values of nitrogen-free extract (N.F.E.), C.P., and C.F.

DISCUSSION

The rape used in this experiment was typical of that grown in Canterbury, New Zealand, as shown by its close similarity in composition, digestibility, and nutritive value to that used in the studies of Lancaster and Adams (1943) and Allison and Thurston (1952).

The significant linear decline of the digestibility of dry matter of rape was small and so of little practical importance. The reasons for this decline are not known, but it is not considered to be due to overmaturity. The mean dry-matter digestibility of selected rape was only 0.4% higher than that of ungrazed rape. This is not in agreement with the results of Hardison *et al.* (1951) for steers, when they found that the mean digestibility of clipped herbage was 91.6% of that of grazed forage. The results of these experiments are in accord, however, with the results of Hardison *et al.* (1954), and of Lofgreen and Meyer (1956), who showed that with steers the digestibilities of grazed and of clipped pasture were similar. Apart from different grazing habits of different animals, differences in digestibility between clipped and grazed herbage would be expected to occur only where species in a mixed sowing or parts of plants of one species differed in digestibility. Differences in digestibility between species are expected, but there is little information on the digestibilities of different parts of the same plant. Hardison *et al.* (1955) have shown differences within lucerne, and the results of Peterson *et al.* (1956) are similar for sudan grass and for lucerne. Many recorded observations lead to the suggestion that stage of maturity and intensity of stocking may be the main factors enabling selective grazing to be expressed as differences in digestibility between clipped and grazed forage. Lack of accord is probably due to stage of maturity and stocking rate, as well as to difference in species and mixtures employed. This provides a reasonable explanation for disagreement between experiments. In the present study the digestibility of N.F.E. and of C.F. was higher, and that of E.Ex. lower, for selected than for ungrazed rape. This is not entirely in accord with the results of Hardison *et al.* (1954). In view of the complex nature of the effect of balance of proximate constituents on their digestibilities it would be unprofitable to discuss possible reasons for this difference. Grazed rape apparently contained more C.F. and ash and less E.Ex. and C.P. than ungrazed rape. This disagrees with the results of Hardison *et al.* (1954) using pasture and lucerne, but is in accord with the results of Bath *et al.* (1956) for ash, C.F., and E.Ex. The results with rape were expected and were entirely consistent with the much greater wastage of leaf blade high in ash, C.P., and E.Ex. - than of stem and petiole which are low in these constituents but high in C.F. (Greenall 1958).

This composition pattern has been previously recorded by Hardison *et al.* (1954) for pasture, by Bath *et al.* (1956) for sudan grass and lucerne, and by Hardison *et al.* (1955) for lucerne. However, with rape the opportunity of the wethers for selective grazing of leaf blade, high in ash, C.P. and E.Ex., was reduced, by its excessive destruction relative to stem and petiole, with the consequent reduction of ash, C.P. and E.Ex. in the forage consumed, to a level lower than that of ungrazed rape.

The divergent results can be reconciled only if it is postulated that wastage in grazing rape was of a different kind and intensity from that occurring with lucerne, sudan grass, and pasture. The observed mechanics of grazing wastage of rape gives support to this hypothesis.

The S.E. value of grazed rape was slightly lower and the nutritive ratio considerably wider than those of ungrazed rape, whereas the reverse occurred with T.D.N., which, however, were only partially corrected for the differences in calorific value of proximate constituents or for fibre percentage. Judged on the bases of T.D.N. the results for rape agree with those of Meyer *et al.* (1956) and Lofgreen and Meyer (1956) for pasture forages. A different situation holds if S.E. provides the basis of comparison. As implied by the method of Lofgreen (1953) for determining T.D.N. of forages other than those high in E.Ex., the organic-matter digestibility is the main determinant of level of T.D.N. Despite large differences in the level of proximate constituents of grazed and ungrazed rape, the organic matter digestibilities of grazed rape and hence its T.D.N. level were slightly higher. However, its food value as evaluated by S.E. and nutritive ratio was slightly lower, its increased digestibility being more than offset by its greater fibre correction.

It is clear that wethers grazed rape selectively in this experiment (see Fig. 1). Whether this was due to a palatability factor, or to accessibility as suggested by Johnston-Wallace (1937) and Peterson *et al.* (1956), or to neglect of over-mature prostrate leaves as suggested by Jones (1933), is undecided. The situation was, however, confused, as leaf blades were destroyed by trampling, by dropping during mastication, and by soiling, much more than were stem and petiole, thus reducing the effect of selective grazing. This was probably further reduced by a stocking intensity equivalent to about ninety sheep per acre. The rape consumed was consequently lower in C.P. and E.Ex. and higher in C.F. than would be expected from a knowledge of the composition of leaf blade, for which the wethers showed preference. The digestibility of selected rape was slightly higher than that of ungrazed rape, so, despite large differences in composition, the food values were similar, though slightly favouring ungrazed rape.

It is concluded that under the conditions of this experiment, which were characteristic of lamb-fattening in Canterbury, grazing had little effect on the nutritive value of rape consumed compared to that offered for grazing.

ACKNOWLEDGEMENTS

I gratefully acknowledge use of digestibility pens and equipment lent by Messrs. W. G. Thurston and R. Belcher of the Grasslands substation, Lincoln. Miss E. Stevenson of Applied Mathematics Laboratory was responsible for statistical analyses of results, and Mr. N. Hood, of the biochemistry section, Crop Research Division, undertook the chemical analyses.

REFERENCES

AHLGREEN, H. L. 1947: *J. Amer. Soc. Agron. 39:* 240-59.

ALLISON, R. M.; THURSTON, W. G. 1952: *N.Z. J. Sci. Tech. A34:* 341-6.

BATH, D. L.; WEIR, W. C., and TORELL, D. T. 1956: *J. Anim. Sci. 15:* 1166-71.

BLAXTER, K. L.; GRAHAM, N. McC.; WAINMAN, F. W. 1956: *Brit. J. Nutr. 10:* 69-76.

DOAK, B. W. 1952: *J. agric. Sci. 42:* 162-71.

GALLUP, W. D.; WHITEHAIR, C. K.; BELL, M. C. 1954: *J. Anim. Sci. 13:* 594-600.

GREENALL, A. F. (in press): *N.Z. J. agric. Res. 1.*

HARDISON, W. A.; LINKOUS, W. N.; WARD, C. Y.; BLASER, R. E.; ENGEL, R. W.; CRAF, G. C. 1955: *J. Anim. Sci. 14:* 1238.

HARDISON, W. A.; REID, J. T.; MARTIN, C. M. 1951: *J. Anim. Sci. 10:* 1070.

HARDISON, W. A.; REID, J. T.; MARTIN, C. M.; WOOLFOLK, P. C. 1954: *J. Dairy Sci. 37:* 89-102.

HEAD, M. J. 1953: *J. agric. Sci. 43:* 281-93.

HOBBS, C. S.; GALLUP, W. D.; TAYLOR, B. R. 1945: *J. Anim. Sci. 4:* 395-402.

JOHNSTON-WALLACE, D. B. 1937: *J. Amer. Soc. Agron. 29:* 441-55.

JONES, M. G. 1933: *Emp. J. exp. Agric. 1:* 361-7.

LANCASTER, R. J.; ADAMS, A. F. R. 1943: *N.Z. J. Sci. Tech. A25:* 131-6.

LOFGREEN, G. P. 1953: *J. Anim. Sci. 12:* 359-65.

LOFGREEN,, G. P.; MEYER, J. H. 1956: *J. Dairy Sci. 39:* 268-73.

MEYER, J. H.; LOFGREEN, G. P.; ITTNER, N. B. 1956: *J. Anim. Sci. 15:* 64-75.

MITCHELL, H. H.; HAMILTON, T. S.; HAINES, W. T. 1940: *J. agric. Res. 61:* 847-64.

PETERSON, M. L.; LOFGREEN, G. P.; MEYER, J. H. 1956: *Agron. J. 48:* 560-3.

RAYMOND, W. F.; KEMP, C. D.; KEMP, A. W.; HARRIS, C. E. 1954: *J. Brit. Grassl. Soc. 9:* 69-76.

SEARS, P. D. 1951: *N.Z. J. Sci. Tech. A33:* 1-29.

TYLER, C. 1950: "Animal Nutrition." Chapman & Hall, London.

WOODMAN, H. E. 1957: *Minist. Agric., London., Bull. 48.*

STUDIES ON THE THICKNESS AND CHEMICAL COMPOSITION OF THE SKIN OF SHEEP

I—DEVELOPMENT TECHNIQUES

By Manika Wodzicka*, Ruakura Animal Research Station,
Department of Agriculture, Hamilton.

(*Received for publication, 11 March 1958*)

Summary

An instrument developed for the rapid and accurate measurement of skin thickness is described. The equipment is accurate to 0.1 mm. Techniques adapted for the determination of the fat and protein contents of the skin of sheep are also described.

Samples were taken from the pelts of three sheep of different live weights to determine the distribution of gradients in skin thickness over the body. A uniform sampling area has been defined. This is near the middle of the back, adjacent to the vertebral column.

General Introduction

Studies of the histology, physiology and biochemistry of the skin of sheep are of interest, not only because of the economic value of leather and wool, but also because skin is the first tissue to come in direct contact with the environment and plays an important part in such vital processes as heat regulation and defence against virus and bacterial invasion.

The experiments described in this and later papers were designed to investigate the problem of the tearing of lamb pelts which occurs in the New Zealand Freezing Works. It is found that some pelts of both the New Zealand Romney Marsh and the New Zealand Southdown × Romney Marsh fat lambs are so thin that during the flaying and fellmongering processes they tear or else become stretched (the latter defect being called the "butcher's strain" fault). The incidence of these two faults is not correlated with the peak employment of unskilled labour in the Freezing Works, but occurs sporadically throughout the season. Therefore, whilst careless butchering and the faulty use of chemicals in the fellmongeries could be responsible for part of the trouble encountered, it seemed likely that an inherent skin weakness might also exist.

Many workers have studied the histological and chemical basis of pelt and leather defects at the end of the fellmongering process and in the finished leather product. The limitations of this approach are, however, considerable. The identity and past history of the animal, are, as a rule, unknown and hence it is frequently difficult to ascertain whether the defects in the product are due to faulty butchering, fellmongering or tanning, or to weaknesses in the living skin. It is believed

---

* Present address: Division of Animal Health and Production, C.S.I.R.O., Sheep Biology Laboratory, Prospect, N.S.W., Australia.

N.Z. J. agric. Res. 1: 582–591.

by the author that a more fruitful line of approach lies in studying the conditions during the life of the animal which may cause skin weaknesses and therefore lead to leather defects. This was the approach adopted in these investigations.

An instrument was developed for the measurement of skin thickness because the torn pelts are characteristically thin. Techniques were adapted for the analysis of the nitrogen, fat, and water content of the skin because these are the three main constituents of skin present in sufficient amounts to be measurable by ordinary chemical methods. Furthermore, the protein fraction of the skin is of interest, because leather is made from the protein fraction.

Gradients in skin thickness over the body of sheep were investigated to determine a uniform sampling area.

Changes in the thickness and chemical composition of the skin of 12 Romney wether lambs were followed from within ten days of birth to five months of age because it was considered desirable, before studying abnormalities, to study these changes as they normally occurred with increasing age.

A study was also made of the effect of shearing on the thickness and chemical composition of the skin of 16 Romney ewes. Freezing Works will not usually accept sheep which have been shorn less than three weeks prior to slaughter. This experiment was therefore designed to study changes which occur in the skin during the first month after shearing.

MEASUREMENT OF SKIN THICKNESS

Before any investigation concerning changes in skin thickness during the growth and adult life of a sheep could be carried out, it was necessary to develop a technique for its rapid and accurate measurement. This involved, not only the design of an instrument which would measure the thickness accurately, but also a sampling method which would ensure:—

(i) removal of the skin from the animal in such a way that comparable cell layers were taken off at each sampling (moreover, it was necessary that these layers should correspond with those removed with the pelt by flaying),

(ii) removal of the wool without altering the skin thickness, cell structure, and chemical composition.

It also seemed desirable to find a method for preserving the skin without altering either its thickness or its chemical composition. This would enable thickness measurements and chemical analysis to be made the day after the samples were taken and permit the sampling of larger numbers of animals per day.

THE INSTRUMENT

Nicov (1931) and Carstens and Kinzelbach (1933) attempted to measure skin thickness in cattle without taking the skin off the animal. Their method consisted of picking up a fold of skin, measuring its

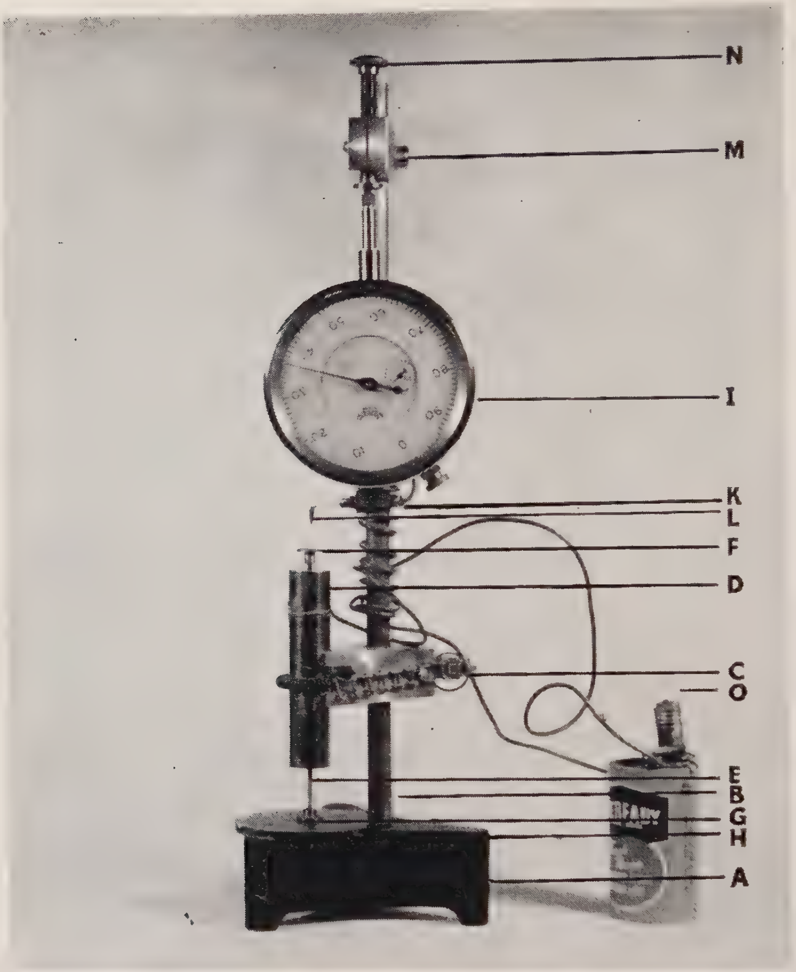


Fig. 1.—The Instrument:

a. A stand.
b. A vertical spindle mounted on the stand.
c. A clamp holding the guide D and fastened to the spindle.
d. A guide for the measuring rod E.
e. The measuring rod which moves free axially but is constrained radially.
f. The top of the rod which carries a small platinum disc forming an electrical contact.
g. The bottom of the rod, which carries another disc of exactly 10 mm. diameter.
h. A platen on which the sample rests.
i. A Mercer dial indicator gauge of the through plunger type, the body of which is clamped to the spindle B.
k. An insulated clamp on the bottom end of the through plunger to which is attached the other electrical contact L.
m. A clamp fastened to the spindle B and tapped for the screw adjustment.
n. An indicator lamp and dry cell connected to contacts L and F.

The weight of the complete measuring rod is 5.9935 g.

thickness under a constant pressure and dividing the result by two, to obtain the thickness. No repeatability figures are given, nor have the authors determined what this measurement represents histologically. Thus, it is not known whether measurements by this method include some subcutaneous tissue as well as the epidermis and the dermis, and whether the nature of the tissue measured varies between measurements and between animals.

Clarke, Stuart, and Frey (1937) measured the thickness of "partly cured lamb skins with the microscope used on sections cut for histological examination." They give no further details of their curing and histological techniques. Since fresh skin was not measured it is impossible to determine the extent of the changes in skin thickness resulting from the curing and the treatment before section cutting. Repeated measurements on the one sample were not made so the accuracy of the method cannot be evaluated.

For the present investigation, the histological techniques involving section cutting were considered to be too laborious, whereas the "pinch" methods described above needed an objective measurement method for comparison before they could be tested and possibly accepted as a valid means for the measurement of skin thickness. A new instrument was therefore designed, in which skin thickness was measured under a small constant pressure. Fig. 1 illustrates this instrument which is henceforth referred to as "the Instrument". The piece of skin to be measured (after being removed from the animal and cleared of wool by the method later described) is placed under a disc weight. The thickness value is recorded on a Mercer spring gauge read when the indicator lamp just lights indicating a closed circuit. The difference between the gauge reading with and without the samples measures the thickness of the skin.

Operation

The measuring rod is allowed to rest with its bottom end e on the platen h. The screw adjustment is turned until the lighting of the indicator lamp indicates that the contacts l and f are touching. The screw adjustment n is then turned back a few turns and the operation repeated to check the zero position.

The screw adjustment is next screwed well up to separate the contact points so that the sample may be inserted under the bottom of the measuring rod g.

The sample is placed on the platen and the measuring rod is allowed to rest on it. By virtue of its diameter and weight, the pressure on the sample will be a constant 0.0763 g per sq. mm.

The screw adjustment is now screwed down until the light indicates that the contacts l and f are touching and the reading on the dial is taken. This reading minus the zero reading will be the thickness of the sample in 0.03 mm. In practice the Instrument readings were accurate to 0.1 mm.

The sample is then removed and the zero checked. A pilot trial (Table 1) showed that the Instrument measurements were highly

TABLE 1. INSTRUMENT MEASUREMENTS OF SKIN THICKNESS: ESTIMATES OF THE COMPONENTS OF VARIANCE

Source of Variation	M.S.	Individuals per subclass	M.S. is an estimate of	Variance Component (mm)
Between pieces*	11.02	20	$\sigma_M^2 + 5\sigma_S^2 + 20\sigma_p^2$	$\sigma_p^2 = 0.005$
Between squares within pieces*	1.12	5	$\sigma_M^2 + 5\sigma_S^2$	$\sigma_S^2 = 0.002$
Between measurements within squares*	0.21	1	σ_M^2	$\sigma_M^2 = 0.002$

* For explanation of the terms "pieces" and "squares" see text.

repeatable. Examination of the frozen sections showed that the amount of subcutaneous tissue removed inadvertently is too small to affect measurement, being less than 0.1 mm. The Instrument operates at a constant pressure, but it was possible that a thicker sample would be less compressed relatively than a thin one. It was essential to determine whether this factor was of importance in the range of thicknesses encountered in the skin of sheep. In Table 2, mean thickness of skin samples measured with the Instrument, immediately after removal from the animal, are compared with eye-piece graticule measurements of frozen sections previously soaked in gum arabic for 24 hours. A close similarity was observed between the means, a correlation of 0.99 being

TABLE 2. COMPARISON OF SKIN THICKNESS MEASUREMENTS WITH THE INSTRUMENT AND WITH THE EYEPIECE GRATICULE

Piece (No.)	Square (No.)	Mean Measurements (mm)		Difference† (mm)
		Instrument	Eye-piece Graticule*	
1	1	2.1	2.2	+0.1
	2	2.0	2.1	+0.1
	3	2.2	2.2	0.0
	4	2.1	2.2	+0.1
	5	2.2	2.3	+0.1
	6	2.2	2.2	0.0
2	1	3.0	3.1	+0.2
	2	2.7	2.8	+0.1
	3	2.8	2.8	0.0
	4	2.9	3.0	+0.1
	5	2.6	2.6	0.0
	6	2.7	2.7	0.0
Mean		2.4	2.5	0.1

* Frozen sections previously kept for 24 hours in gum arabic.
† Difference between eye-piece graticule and instrument measurements.

obtained between measurements by the Instrument and those with the eye-piece graticule. A similar correlation was obtained for both the thinner and the thicker samples. Therefore, in terms of the requirements outlined at the beginning of this section, the Instrument was shown to be satisfactory.

PREPARATION OF SAMPLES AND MAKING OF MEASUREMENTS

The sample normally consisted of a piece 2 × 2 cm. Before removal this was marked out into four 1–cm squares. The piece of skin removed was always slightly bigger than the area marked out to allow for the unevenness in thickness at the edges caused by the cutting-out operation. It was removed with the scalpel, then cleaned of subcutaneous tissue and the blood was washed off with water. The sample was dried with filter paper and placed in a tightly stoppered jar. It was then put into a refrigerator for 24 hours. The wool was subsequently removed by shaving with a blade razor and skin thickness measured with the Instrument. The skin was then replaced in the jar for chemical analysis.

The thickness of each square was measured five times so that 20 measurements were made on each piece. Throughout this work, except for the data of Table 2 the "mean" for skin thickness is the mean of these 20 measurements.

In Table 2 the pieces were larger, each consisting of six squares arranged 3 × 2. (In variance analyses means were used in the calculations.)

Preliminary experiments showed that the blade razor used while the skin was on the animal or after it had been taken off, was a satisfactory method for wool removal without altering the nature of the skin (Table 3).

TABLE 3. EFFECT ON SKIN THICKNESS OF SHAVING BEFORE AND AFTER REMOVAL OF THE SAMPLE FROM THE ANIMAL

Shaven before		Shaven after	
Piece No.	Mean (mm)	Piece No.	Mean (mm)
1	1.8	3	1.9
2	1.9	4	2.0
Mean	1.8		1.9

It was also found that skin could be preserved in a refrigerator (at 4°c.) without altering in thickness (Table 4), but that it increased in thickness when kept in a deep freeze (at —15°c.).

Nitrogen was determined by the Kjeldahl method. The conventional factor of 6.25 was used to convert nitrogen to protein. The

TABLE 4. THE EFFECT ON SKIN THICKNESS OF STORAGE FOR 24 HOURS IN A REFRIGERATOR

Piece No.	Fresh	After Storage
	Mean (mm)	Mean (mm)
1	1.8	1.9
2	1.9	1.9
3	1.9	1.8
4	2.0	2.0
Mean	1.9	1.9

protein content is of course an approximation, since the factor is not necessarily correct for skin, and nitrogen compounds other than protein are present.

For fat determination, the samples were dehydrated with ethyl alcohol and dried in an oven. They were then extracted with petroleum ether.

Dry matter was taken as the sum of the fat-free residue and the ether extract.

Examination of frozen sections showed that careful removal of the skin from the animal with a scalpel results in uniform samples, consisting of the dermis and the epidermis, which corresponds to what is removed with the pelt at slaughter.

VARIATION OF SKIN THICKNESS OVER THE BODY OF A SHEEP

This part of the investigation was designed to find out how skin thickness varied over the body of a sheep and to identify a uniform sampling area. Such an area is desirable in a study involving serial sampling in order to prevent the confounding of treatment differences with positional variations. The pelts of three sheep with widely different live weights were sampled once in 20 positions, and the thickness of the samples was determined. Animals with a wide disparity in size were chosen to discover whether the gradients in skin thickness were altered with size.

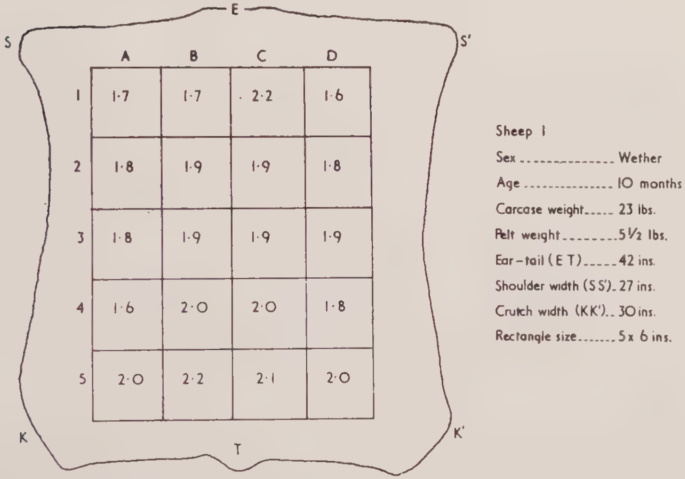
RESULTS

The means of the measurements of skin thickness are shown in Figs. 2, 3, and 4. The carcass and pelt weights and the pelt dimension measurements appear at the side of each of these figures. Results obtained indicate that:—

1. There was a steep dorso-ventral gradient in skin thickness on either side of the median dorsal line, which showed a high degree of bilateral symmetry; the skin was thicker near the vertebral column;

2. The skin was thicker near the tail along the back of the sheep and, in the oldest sheep, also towards the neck:

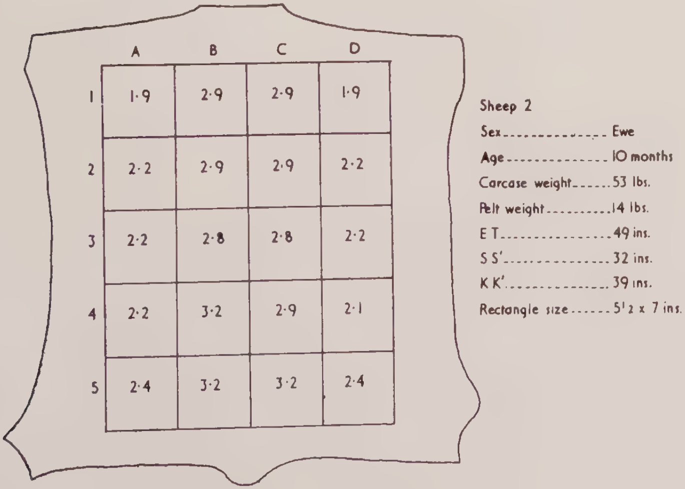
3. There was an area of uniform skin thickness, in the middle of the back, on either side of and parallel to the vertebral column, which is suggested as a suitable sampling area.



	A	B	C	D
1	1·7	1·7	2·2	1·6
2	1·8	1·9	1·9	1·8
3	1·8	1·9	1·9	1·9
4	1·6	2·0	2·0	1·8
5	2·0	2·2	2·1	2·0

Sheep 1
Sex................Wether
Age................10 months
Carcase weight......23 lbs.
Pelt weight..........5½ lbs.
Ear–tail (E T)......42 ins.
Shoulder width (S S')..27 ins.
Crutch width (KK')..30 ins.
Rectangle size.......5 x 6 ins.

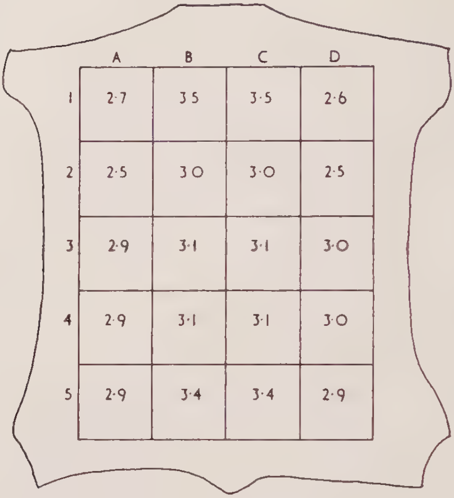
Fig. 2.—Distribution of skin thickness gradients over the body of a sheep. Means in mm.



	A	B	C	D
1	1·9	2·9	2·9	1·9
2	2·2	2·9	2·9	2·2
3	2·2	2·8	2·8	2·2
4	2·2	3·2	2·9	2·1
5	2·4	3·2	3·2	2·4

Sheep 2
Sex................Ewe
Age................10 months
Carcase weight.......53 lbs.
Pelt weight...........14 lbs.
E T.................49 ins.
S S'................32 ins.
K K'................39 ins.
Rectangle size.......5½ x 7 ins.

Fig. 3.—Distribution of skin thickness gradients over the body of a sheep. Means in mm.



	A	B	C	D
1	2·7	3·5	3·5	2·6
2	2·5	3·0	3·0	2·5
3	2·9	3·1	3·1	3·0
4	2·9	3·1	3·1	3·0
5	2·9	3·4	3·4	2·9

Sheep 3

Sex	Wether
Age	3 years
Carcase weight	72 lbs
Pelt weight	15 lbs.
E T	58 ins.
S S′	38 ins.
K K′	48 ins.
Rectangle size	7 x 8 ins.

Fig. 4.—Distribution of skin thickness gradients over the body of a sheep. Means in mm.

DISCUSSION

The Instrument used in these experiments is accurate, quick and easy to use. It is useful for experiments where part of the skin sample is used for histological sections and part for chemical analyses, since both of these require the removal of the skin from the animal. Also, a relatively large sample is required for chemical analysis, being large enough for measurement with the Instrument. For this, at least one square centimetre of skin has to be removed. Experiments described in this series of papers indicate that the health of the animals appears to be unaffected even by the removal of four samples of 4 sq. cm each, within a period of one month. However, for large scale work, this method is disadvantageous because there is some reduction in both pelt and fleece values and because the number of samplings must be limited in number and frequency.

Where skin thickness values alone are required, a "pinch" type of instrument similar to those used by Nicov (1931) and Carstens and Kinzelbach (1933), should it prove an accurate method, would be preferable, since the skin would not have to be removed from the animal. Even where changes in the histological structure of the skin are being investigated as well, the "pinch" method would be useful, because the amount of skin required for histological sections is only of the order of a few sq. mm. Thus, the use of this method would make it possible to follow changes in thickness and histology in the skin of the same animal at frequent intervals.

The technique for measurement of skin thickness, used in these investigations, could also be used in future work, to test the validity of other instruments used for the same purpose.

The general pattern of skin-thickness gradients was similar in all three sheep, with the exception of the thicker neck region in the oldest animal. Since one ewe and two wethers, a total of three animals only, were used, the results should, however, be treated with caution.

The pattern of skin-thickness gradients in lambs of both sexes from birth to maturity and that of adults at different times of the year and under varying environmental conditions should be investigated. If the ratios of skin thickness between the different parts of the body remain constant at all stages of growth, and under all environmental conditions, then another sampling area in the less valuable part of the pelt would be preferable. A sample, for example taken from the lateral edge of the pelt (belly), would not damage the pelt to the same extent as one removed from the middle of the back. If, on the other hand, the gradients, or at least their steepness, vary at different stages of a sheep's development and under different environmental conditions, it would be interesting to see which areas are most affected.

The main dorso-ventral gradient in skin thickness is similar to the one found by Carter (1943) in the density of the follicle population of the adult Merino, more follicles occurring in the areas of thicker skin. Carter also found a weak antero-posterior gradient which was not found in these studies of skin thickness. However, he found that in one sheep out of the six studied, there was no antero-posterior gradient, but two well separated areas of high density along the back, one antero-thoracic and the other lumbo-sacral, i.e., similar to the one encountered in sheep 3.

REFERENCES

Carstens, P.; Kinzelbach, W. 1933: *Zuchtungskunde 8:* 135.
Carter, H. B. 1943: *Commonw. sci. industr. Res. Org., Melbourne, Bull. 164.*
Clarke, T. D.; Stuart, L. S.; Frey R. W. 1937: *Sonderdr. Stiasny Festschrift, 144.*
Nicov, T. 1931: *Zeitschr. f. Zucht. B21:* 351.

STUDIES ON THE THICKNESS AND CHEMICAL COMPOSITION OF THE SKIN OF SHEEP

II—VARIATIONS DURING GROWTH

By MANIKA WODZICKA*, Ruakura Animal Research Station,
Department of Agriculture, Hamilton

(*Received for publication, 11 March 1958*)

Summary

Changes in the thickness and chemical composition of the skin in sheep have been followed from 10 days after birth to five months of age.

Skin increased in thickness by some 14% until the lambs were 5 to 10 weeks of age. By 13 weeks it decreased to the original thickness at birth and remained at this value until 5 months of age.

The fat content of the skin increased with age, but the nitrogen content remained constant. The dry-matter content increased with age.

It was found that the skin of the two slower-growing lambs was thinner and had a lower fat content than that of the remainder.

EXPERIMENTAL PLAN

Twelve Romney wether lambs were sampled at six positions within 10 days of birth and at monthly intervals thereafter. Each sample consisted of a square, 4 sq. cm in area. The sampling positions were chosen on the basis of results described previously and their location on the body of the animal is outlined in Fig. 1. At each sampling, skin thickness was measured, and the fat and nitrogen content of the samples determined. Dry matter was calculated. The lambs were weighed at each sampling. In addition, the staple lengths of wool grown post-natally, in a position half-way up the side of the animal, were measured. On the last occasion, samples of wool were taken from the same position as for the staple-length measurement. The wool count or quality was determined and the benzol test for hairiness (McMahon 1937) carried out. These additional measurements were obtained to see whether they were related to the skin characteristics investigated.

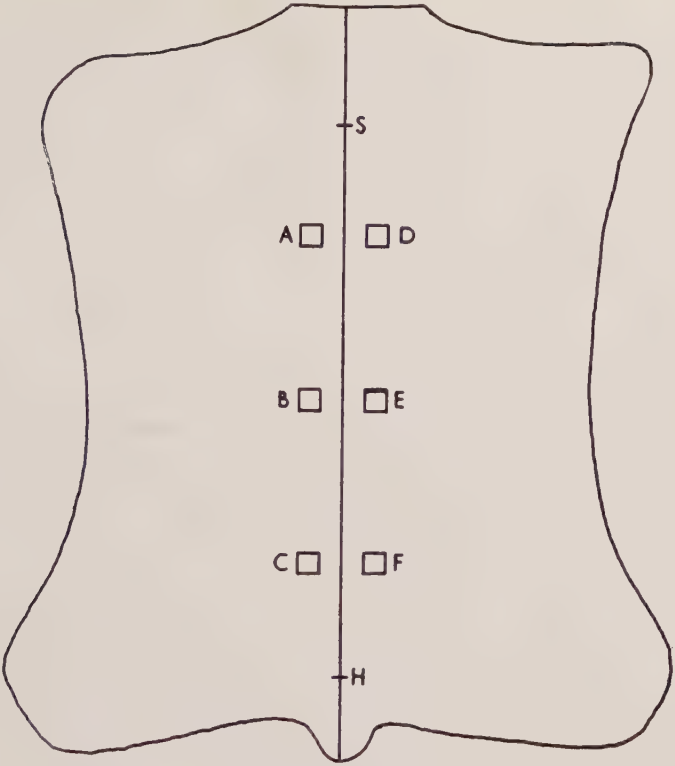
Since it was not known whether skin thickness and chemical composition changed rapidly after birth, the first samples should have been taken at birth. Unfortunately, however, the animals were not available at lambing and therefore the first sampling took place one to ten days after birth.

The uniform sampling area decided upon was based on work with pelts and it was found difficult to define the precise positions in the living animal. Moreover, it was uncertain that the same gradients in skin thickness occur in lambs as in older animals. Thus, two types of variation could confound time trends in skin thickness and chemical

---

* Present address: Division of Animal Health and Production, C.S.I.R.O., Sheep Biology Laboratory, Prospect, N.S.W., Australia.

N.Z. J. agric. Res. 1: 592–600.



S Vertebral column at the shoulder level

H Vertebral column at the hip-bone level

SM = HM

A Sample situated at 1/3 of the distance SH posterior to S and one inch to the left of the vertebral column.

B Sample situated at 1/2 of the distance SH posterior to S and one inch to the left of the vertebral column.

C Sample situated at 1/3 of the distance SH anterior to H and one inch to the left of the vertebral column.

D E & F Samples similarly situated to A B & C but on the right of the vertebral column.

Fig. 1.—Sampling positions used.

composition: that due to the differences between the lambs and that due to differences between positions. Two 6 × 6 Latin squares were, therefore, used to obtain error control in two directions.

Experimental Animals

The 12 experimental lambs came from the Ruakura Fertility flock. The ewes and lambs used in this experiment were run separately from the rest but under similar (i.e., grassland fat-lamb) conditions. Like the rest of the flock, they were docked at approximately three weeks of age and weaned on 9 December. Table 1 shows that all lambs except lamb 6 were born between 12 and 30 August. Lamb 6 was a month younger than the rest of the animals, being a replacement for a lamb that died.

TABLE 1. DETAILS OF LAMBS USED

Lamb No.	Age of Ewe (years)	Birth date of Lamb (1952)	Comment
1	2	12 Aug.	Good lamb
2	2	13 Aug.	Poor lamb with undershot jaw.
3	2	16 Aug.	Good lamb.
4	2	18 Aug.	Good lamb.
5	2	18 Aug.	Good lamb, developed septic eye 20.12.52 and lost some weight.
6*	3	24 Sep.	Good lamb—replacement for Lamb 6A.
7	4	20 Aug.	Good lamb.
8	2	23 Aug.	Poor lamb, ewe bad milker.
9*	2	23 Aug.	Good lamb.
10	2	23 Aug.	Good lamb.
11	6	27 Aug.	Good lamb, died 2.1.53, suspected cause, pulpy kidney.
12	3	30 Aug.	Good lamb.
6A	2	24 Aug.	Died 20.9.53.

* From the Ruakura Hill Country Station flock.

Lamb 10 died after the fifth sampling: the suspected cause was pulpy kidney. Lambs 2 and 8 grew at a slower rate than the other animals. Lamb 2 developed within the first six weeks of its life a very undershot jaw; the mother of lamb 8 appeared to be a bad milker and dried up completely within two months after lambing. Lamb 5 developed an infected eye following an injury shortly after the fourth sampling. Though treated, it lost the sight of the eye concerned and its growth curve shows a check at that point.

Fig. 2 shows that, in spite of being handled twice a month, and subject once a month to an operation and a separation from their mothers, lasting two to four hours, the lambs' growth curves remained normal. Lambs 2 and 8 grew more slowly, but causes other than those due to the experimental procedure are considered to be responsible. At weaning time, these 12 lambs averaged 59.0 lb whereas the wether lambs of the whole Fertility flock averaged 57.5 lb.

Although samples were relatively large, the lambs suckled or grazed immediately after the operation, showing no discomfort. In no case was trouble experienced with the healing of wounds. A month after sampling, the clipped area and scar were the only signs of the operation. After three months, the wool had to be completely shaved before the scar marking the site of the operation could be located.

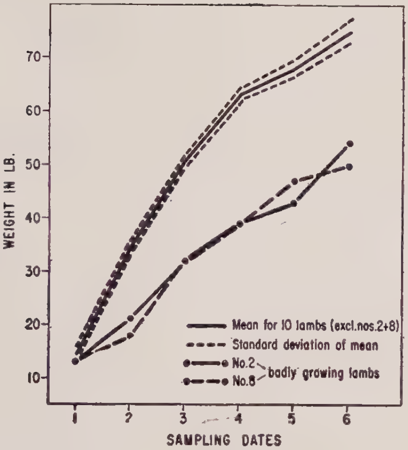


Fig. 2

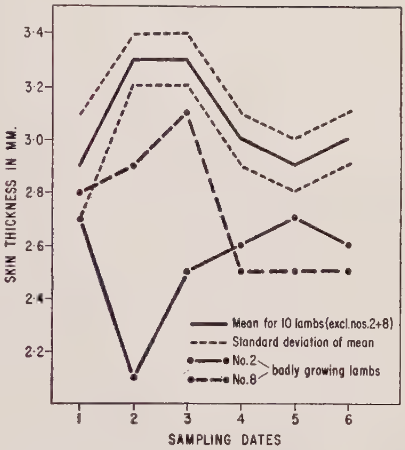


Fig. 3

Fig. 2.—Growth of lambs.
Fig. 3.—Skin thickness measurements.

RESULTS

Skin Thickness

Fig. 3 shows that skin increased slightly in thickess from the first sampling (1 to 10 days after birth) to the second sampling (30 to 40 days after birth). After the third sampling (8 to 10 weeks after birth), it decreased to a value similar to the one obtained at the first sampling. From the fourth sampling (12 to 14 weeks after birth) until the end of the experiment, when the animals were approximately 22 weeks of age, the skin thickness remained unchanged. The analysis of variance (Table 2) shows that there were significant differences between sampling dates. Fig. 3 shows that lambs 2 and 8, which grew more slowly, had the thinnest skins.

TABLE 2. ANALYSIS OF VARIANCE OF CHANGE IN SKIN THICKNESS
(For convenience, all calculations for the analysis of variance are in tenths of a millimetre)

Source of Variation	d.f.	M.S.	F.	Level of Significance
Between Latin squares	1	0.65	1	N.S.
Between lambs within Latin squares	10	39.25	6.24	**
Between positions	5	39.21	6.23	**
Positions × Latin squares	5	1.28	1	N.S.
Between sampling dates	5	31.25	4.97	**
Sampling dates × Latin squares	5	18.91	3.01	*
Error	39	6.29		
Total	70			

N.S. Not significant.
* Significant at 5% level.
** Significant at 1% level.

There was no correlation between skin thickness and age, live weight, or live-weight gain because while skin initially increased in thickness, then decreased and finally remained at the same value until the end of the experiment, the other measurements continued to increase throughout.

In Table 3, correlations within sampling dates between skin thickness and age, and skin thickness and growth (as measured by live weight, live-weight gain and relative growth rate), are given. Correlations, which were significant or which approached significance were shown to exist between skin thickness and live weight and skin thickness and live-weight gain. The "z" test was used to test the hypothesis that each of these sets of correlations came from the same population and could be combined into an estimate of an overall correlation. It was concluded that there was no reason to suspect that the six correlations in each group might not have been drawn from the same population. The average correlation coefficient between skin thickness and live weight was 0.707, and the correlation coefficient between skin thickness and live-weight gain +0.799, both these values being significant at the 1% level of significance. There was no significant correlation between skin thickness and age except at the first sampling.

TABLE 3. CORRELATIONS BETWEEN SKIN THICKNESS AND GROWTH WITHIN SAMPLING DATES

Sampling Date	d.f.	Thickness: Age	Thickness: Live Weight	Thickness: Live-weight Gain	Thickness: Relative Growth Rate
1	10	0.668*	0.929**	0.818**	0.219 N.S.
2	10	0.374 N.S.	0.678*	0.524 N.S.	0.248 N.S.
3	10	0.380 N.S.	0.508 N.S.	0.723**	0.339 N.S.
4	10	0.360 N.S.	0.780**	0.818**	0.569 N.S.
5	10	0.109 N.S.	0.446 N.S.	0.967**	0.194 N.S.
6	9	0.254 N.S.	0.778**	0.551 N.S.	0.360 N.S.

N.S. Not significant.
* Significant at 5% level.
** Significant at 1% level.

There were no significant correlations between skin thickness and wool-staple length, count, or percentage hairiness.

The data of Table 2 showed that there were significant differences in skin thickness due to positions. However, there were no significant gradients in the positions sampled. It is suggested that a smaller sampling area closer to the centre of the back could be more efficient.

Chemical Composition

Changes, during the experimental period, in the fat, calculated protein, and dry-matter contents of the skin are shown in Figs. 4, 5, and 6.

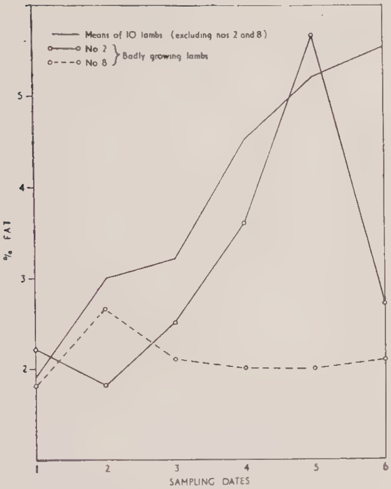


Fig. 4

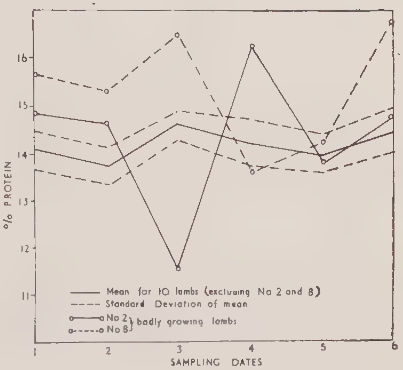


Fig. 5

Fig. 4.—Fat content expressed as percentage of the original weight.

Fig. 5.—Calculated protein content expressed as percentage of the original weight.

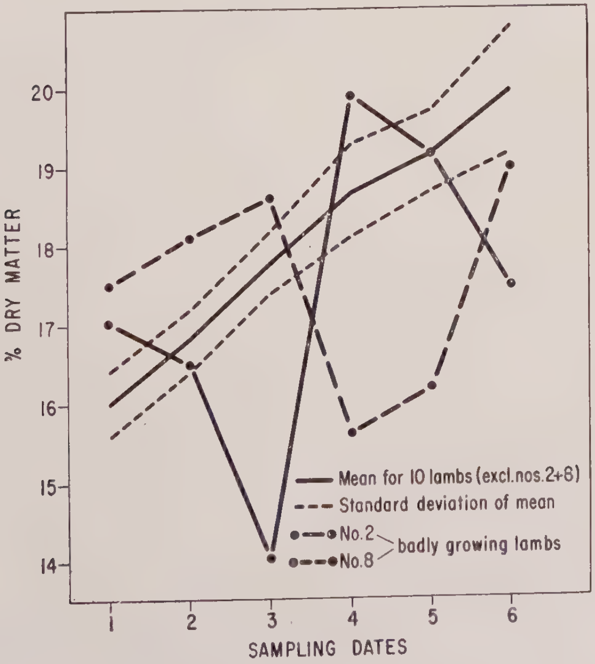


Fig. 6.—Dry-matter content expressed as percentage of the original weight.

The calculated protein and dry-matter percentages were analysed as such because they did not appear to violate the conditions required for the analysis of variance. Bartlett's test, in particular, showed that the variances were homogeneous. Logarithmic transformations were used on the fat-percentage data in which the variances increased from the first to the last sampling.

The fat content of the skin increased with age and the analysis of variance (Table 4) showed this increase to be real. Lambs 2 and 8, which grew more slowly than the others, had skins with a lower fat content; also there was a correlation (significant at the 1% level) between fat content and live weight over the whole experimental period.

TABLE 4. ANALYSIS OF VARIANCE OF THE CHANGE IN FAT CONTENT OF THE SKIN

(Percentages transformed into logarithms for all calculations in this table)

Source of Variation	d.f.	M.S.	F.	Level of Significance
Between Latin Squares	1	0.0030	1	N.S.
Between lambs within Latin squares	10	0.1059	21.61	**
Between positions within Latin squares	10	0.0254	5.18	**
Between dates	5	0.2604	53.14	**
Dates × Latin squares	5	0.0129	2.63	*
Error	39	0.0049		
Total	70			

N.S. Not significant.
* Significant at 5% level.
** Significant at 1% level.

On the other hand, the nitrogen content of the skin did not alter appreciably, nor did the mean nitrogen content of the skin of the two poorly-growing lambs differ from that of the other ten.

Since the fat content rose with age and in this experiment the dry matter consisted of fat plus the estimated protein content, the dry-matter content rose with increasing age.

A close agreement of the values for the protein content of the skin, calculated by multiplying the nitrogen values obtained by the Kjeldahl method by 6.25 and those obtained by weighing the fat-free residue, was observed. A coefficient of correlation calculated on 100 samples was of the order of +0.94. A regression equation was calculated:

$$Y = 15.23 + 1.05 (X - 15.32)$$

Where Y^* = nitrogen × 6.25 and

X = dried, fat-free residue. On the basis of these results, it could be assumed that the dried, fat-free residue was equivalent to nitrogen multiplied by 6.25. Since minerals must have been present the nitrogen factor must be too high to represent truly the protein and other nitrogenous compounds.

---

* Kjeldahl.

DISCUSSION

From the results of this experiment it appears that within 10 days of birth, the skin of a lamb is as thick as at five months of age. Unfortunately, no measurement was obtained at birth and it is possible, therefore, that the high value obtained at the first sampling was the result of a rapid increase in skin thickness. The correlation (significant at the 1% level) between skin thickness and age at the first sampling suggests that this may take place.

Between 1 and 4–10 weeks after birth, skin increased in thickness by approximately 14%. By 13 weeks of age it had decreased to the value obtained at the first sampling and, until the end of the experiment at about five months of age, it did not alter in thickness. A study of the changes in the histological structure of the skin occurring during that period, together with a more precise definition of the shape of the post-natal curve of skin thickness, is necessary before an explanation of these changes can be more than speculative.

Changes in the wool follicle and fibre populations in the Romney breed occurring during the early post-natal period have been studied by several workers. Burns (1949) found that the initiation of the secondary follicles stopped at approximately three months of age. Her results, however, must be treated with caution because they are based on studies of two lambs only, both with poor growth curves, only one surviving more than six months after birth. Since, moreover, she sampled at three monthly intervals, the period of follicle initiation is ill-defined. Similar time relationships were, however, found by Goot (1945). Henderson (1953), working on fibre populations of 12 lambs and sampling four weeks after birth and at eight weekly intervals thereafter, found that the major increase in fibre numbers took place before 12 weeks of age.

It is possible that both follicle formation and skin thickness are a reflection of the physiological activity of the skin and that a peak of this activity is reached 5 to 12 weeks after birth. Alternatively these changes could be linked with a varying nutritional status of the lambs, but the growth curve showed no check or "boost" at any point to support this view.

Another explanation of the rise and decline in skin thickness within the first five months after birth could be derived from the results obtained in a shearing experiment to be described later which indicate that skin increases markedly in thickness after shearing. It is possible that skin responds to changes in the environmental temperature by changes in thickness, and that the increased thickness after shearing is caused by a lowering of the skin temperature following the removal of wool. If this hypothesis is true, then the expulsion of the lamb from the uterus into a colder environment could lead to an increase in skin thickness. Then, as the length of wool increased and the environmental temperature rose with the approaching summer, skin thickness would fall to a lower value. Again, a more precise definition of the skin thickness curve and of the histological picture, together with a study of the effect of the changes in the environmental temperature on skin temperature and thickness should throw some light on this [illegible]. It is difficult, however, to suggest a reasonable physiological explanation for the dependence of skin thickness on thermal environment.

Of interest in connection with the problem of tearing pelts is the fact that the two lambs which grew more slowly, had thinner skins throughout. These results are in agreement with those of Clarke *et al.* (1937), who found that in a post-weaning study of nine pairs of Rambouillet lambs on widely different planes of nutrition, the underfed lambs had skins about 57% of the thickness of the fully fed ones. It must be remembered that their methods of measurement are open to criticism, whereas conclusions based on the few animals used in this experiment must necessarily be regarded as tentative, but it would seem that slow growth prior to slaughter could produce pelts which were liable to tearing. A study of the effects of widely differing planes of nutrition on the thickness, histology, and chemical composition of the skin in lambs would elucidate the importance of the nutritional factor.

The nitrogen content of the skin remained constant during the entire experimental period, but the fat content rose with age. The skin of the two poorly-growing lambs had lower fat values throughout. Their nitrogen content, however, was similar to that obtained for the remainder of the experimental group. These results confirm the findings of Clarke *et al.* (1937), who found that underfed lambs had skins with a lower fat content. They are also in conformity with the results of such workers as Hammond (1932), Wallace (1948) and Palsson and Verges (1952), who found that the fat content of tissues in the carcass of sheep increased with age. It would be of interest to know whether torn pelts have a lower fat content than the normal ones and to clarify the factors responsible for their low tensile strength.

REFERENCES

BURNS, M. 1949: *J. agric. Sci. 39:* 64.

CLARKE, T. D.; STUART, L. S.; FREY, R. W. 1937: *Sonderdr. Stiasny Festschrift, 144.*

GOOT, H. 1945: *N.Z. J. Sci. Tech. A27:* 173.

HAMMOND, J. 1932: "Growth and Development of Mutton Qualities in the Sheep", Oliver Boyd, Edinburgh.

HENDERSON, A. E. 1953: *J. agric. Sci. 43:* 12.

MCMAHON, P. R. 1937: *J. Text. Inst. 28:* T349.

PALSSON, H.; VERGES, J. B. 1952: *J. agric. Sci. 42:* 1.

WALLACE, L. R. 1948: *J. agric. Sci. 38:* 243.

STUDIES OF THE THICKNESS AND CHEMICAL COMPOSITION OF THE SKIN OF SHEEP

III—EFFECT OF SHEARING

By MANIKA WODZICKA,* Ruakura Animal Research Station,
Department of Agriculture, Hamilton.

(*Received for publication, 11 March, 1958*)

Summary

Changes in the thickness and chemical composition of the skin following shearing were studied in 16 Romney ewes. The results obtained indicated that skin increased considerably in thickness after shearing, although (because of the limited number of samplings) the exact magnitude, speed, and duration of response, were unknown. The chemical composition of the skin remained constant after shearing.

INTRODUCTION

The general purpose of these investigations has been described in the first paper of this series. In this experiment, the effect of shearing on the thickness and chemical composition of the skin was studied in 16 Romney ewes.

EXPERIMENTAL PLAN

Sixteen Romney ewes were sampled:

2 days before shearing, on 6 January 1953
2 days after shearing, on 10 January 1953
14 days after shearing, on 22 January 1953
31 days after shearing, on 8 February 1953

Each sample consisted of a square, 4 sq. cm in area. Skin thickness measurements were done at each sampling, by the method described in the first paper of this series. The sampling positions were taken closer to the middle of the back of the sheep to reduce the positional variance (Fig. 1).

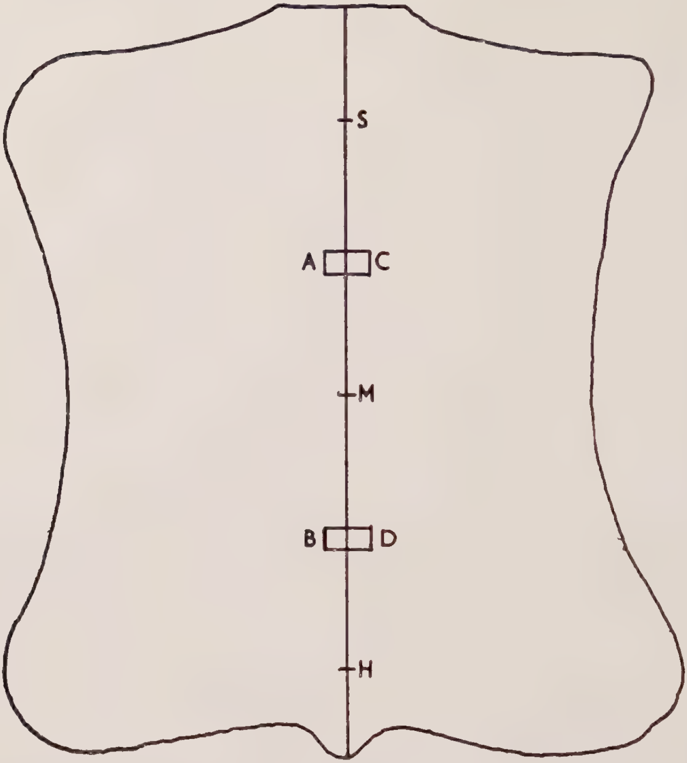
Nevertheless, a Latin-square design consisting of four 4 × 4 Latin squares, was used to prevent the possible confounding of the treatment effects by positional trends.

A possible weakness of the design lay in the fact that only one sample was taken prior to shearing, because the animals became available only a few days before the date. However, as already shown, even in growing lambs, skin thickness varied but little over long periods. It was therefore assumed that, prior to shearing, skin thickness remained constant and that any changes following the first sampling were due to the effect of shearing.

---

* Present address: Division of Animal Health and Production, C.S.I.R.O., Sheep Biology Laboratory, Prospect, N.S.W., Australia.

N.Z. J. agric. Res. 1: 601–606.



S Vertebral column at the shoulder level.

H Vertebral column at the hip-bone level.

SM = HM

A Sample situated half way between S and M to the left of and adjacent to the vertebral column.

B Sample situated half way between H and M to the left of and adjacent to the vertebral column.

C & D Samples similarly situated to A and B but to the right of the vertebral column.

Fig. 1.—Sampling positions used.

Since it was found that the dried, fat-free residue was equivalent to nitrogen multiplied by 6.25, the weight of the fat-free residue was called protein and no Kjeldahl analyses were done.

RESULTS

Skin Thickness

Skin increased temporarily in thickness after shearing (Fig. 2). The analysis of variance (Table 1) shows that this difference was significant at the 1% level.

TABLE 1. ANALYSIS OF VARIANCE OF THE CHANGE IN SKIN THICKNESS
(For convenience, all calculations for the analysis of variance are in tenths of a millimetre.)

Source of Variation	d.f.	M.S.	F.	Level of Significance
Between Latin squares	3	28.13	4.06	*
Between sheep within Latin squares	12	43.56	6.30	**
Between positions within Latin squares	12	6.45	1	N.S.
Between dates	3	183.79	26.59	**
Dates × Latin squares	9	12.22	1.77	N.S.
Error	24	6.91		
Total	70			

N.S. not significant.
* significant at 5% level.
** significant at 1% level.

Because of the limited number of samplings, the exact magnitude, speed, and duration of this response are not known, but the change was evident 48 hours after shearing and had passed its maximum value by 31 days. Table 2 shows that the mean increase in thickness expressed as percentage of the pre-shearing value was 10.2% at 2 days 28.5% at 14 days, and 15.9% at 31 days after shearing. The maximum increase in skin thickness varied from 12.9% to 80.6% of the original

TABLE 2. INCREASE IN SKIN THICKNESS AFTER SHEARING EXPRESSED AS A PERCENTAGE OF THE THICKNESS BEFORE SHEARING

Sheep No.	2 Days after Shearing	14 Days after Shearing	31 Days after Shearing
1	3.4	31.0	20.7
2	19.2	23.1	7.7
3	7.1	17.8	19.7
4	14.3	25.0	25.0
5	6.1	42.4	1.4
6	19.3	80.6	16.1
7	3.4	20.7	3.4
8	16.6	20.8	25.0
9	17.2	37.9	20.7
10	3.8	26.9	26.9
11	13.3	30.0	23.3
12	13.3	20.0	20.0
13	12.9	25.8	19.3
14	3.1	18.7	0.0
15	3.2	22.6	16.1
16	6.5	12.9	9.7
Mean	10.2	28.5	15.9

value in the individual sheep. Furthermore, while the maximum response in most sheep occurred at the sampling taken 14 days after shearing, in two sheep it was greatest at the last sampling, while two other sheep showed an equal response at both samplings. In other words, sheep varied in the magnitude and speed of the response. The increase at 14 days after shearing was about twice that between the first and second sampling in the experiment on lambs reported in Part II of this series.

Comparison of the value of the ewes prior to shearing (Fig. 2) with that for lambs less than 10 days old, shows that the thickness was almost the same. The two groups of animals were not strictly comparable, for the sex was different. Moreover, the skin thickness of ewes may depend upon nutritional status and other factors in their previous history, so these pre-shearing values are not necessarily standard. However, it does seem probable that there is no marked increase in skin thickness with age.

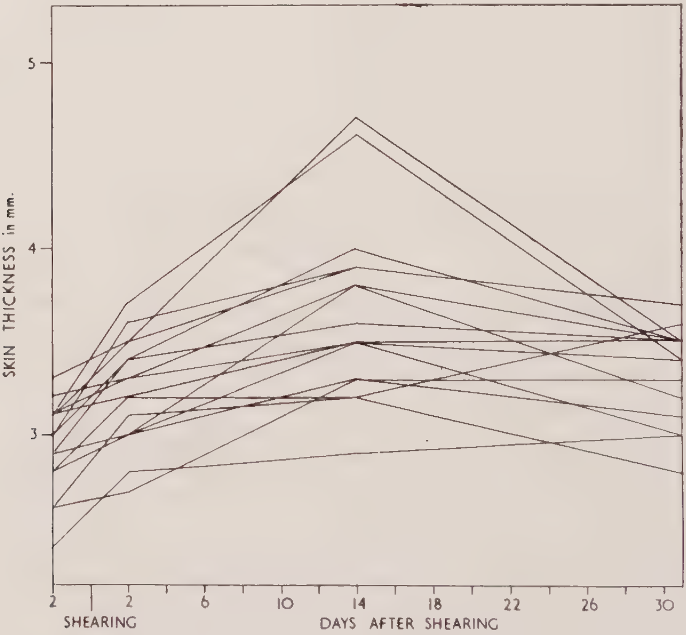


Fig. 2.—Individual skin thickness measurements.

There were no significant positional differences in skin thickness indicating that the area sampled was uniform in thickness.

Chemical Composition

Tables 3 and 4 show that there was no appreciable change in the chemical composition after shearing. The mean calculated protein was 16.6%, 16%, 16.9% and 16.4% at the four samplings. The mean fat content was 7.1%, 5.6%, 6.9%, and 5.5% respectively. The

TABLE 3. ANALYSIS OF VARIANCE OF THE EFFECT OF SHEARING ON THE FAT CONTENT OF THE SKIN EXPRESSED AS PER CENT OF THE ORIGINAL WEIGHT

Source of Variation	d.f.	M.S.	F.	Level of Significance
Between Latin squares	3	45.36	18.44	**
Between sheep within Latin squares	12	48.53	19.73	**
Between positions within Latin squares	12	2.80	1.14	N.S.
Between dates within Latin squares	12	5.33	2.17	N.S.
Error	24	2.46		
Total	63			

N.S. not significant.
* significant at 5% level.
** significant at 1% level.

TABLE 4. ANALYSIS OF VARIANCE OF THE EFFECT OF SHEARING ON THE PROTEIN CONTENT OF SKIN EXPRESSED AS PER CENT OF THE ORIGINAL WEIGHT

Source of Variation	d.f.	M.S.	F.	Level of Significance
Between Latin squares	3	8.95	4.81	**
Between sheep within Latin squares	12	7.89	4.24	**
Between positions within Latin squares	12	1.69	1	N.S.
Between dates within Latin squares	12	1.74	1	N.S.
Error	24	1.86		
Total	63			

N.S. not significant.
* significant at 5% level.
** significant at 1% level.

calculated protein was higher than for the lambs in the growth experiment (Part II) which had a mean of 14.2% over the experimental period. The fat content of the skin of the ewes was also slightly higher than that of the lambs (mean 4.6% at five months of age). The dry-matter content of the skin in ewes was, therefore, higher than that of the lambs.

DISCUSSION

A study of the changes in the histological structure of the skin after shearing, a more precise definition of the post-shearing changes in skin thickness, and information on the influence of effective environmental temperatures, seem necessary before any theory explaining this increase in skin thickness can be more than speculative. An experiment in which shorn and unshorn groups of sheep are subjected to different environmental temperatures might supply an answer to this question.

In connection with the problem of tearing of lamb pelts, it should be noted that if the skin of lambs also increases in thickness after shearing, recently shorn lambs would tend to have thicker pelts than unshorn ones, and might yield better pelts.

Some points of general interest arise from the results of this and the experiments previously described. First, the large differences between sheep, in the characteristics studied, emphasise the usefulness of an experimental approach where the animal is studied over a long period and used as its own control. In experiments where groups of animals are slaughtered at different points in time, although a more complete set of measurements can be obtained for each group at a given moment, changes occurring between two slaughter dates may be obscured by large variations between animals.

Secondly, these investigations have emphasised the scarcity of existing knowledge of the histological and physiological changes (other than those connected with the wool follicle and fibre) occurring in the skin of sheep during growth, and later in adult life. Such knowledge is essential if conditions causing skin weakness and leading to pelt and leather defects are to be understood and, if possible, eliminated.

Leather characteristics are the result of both the nature of the original product, skin, and the fellmongering and tanning processes. It is improvements in the quality of the original product that lie within the province of the animal producer. With this aim in mind, knowledge of the histology, physiology, and biochemistry of the skin should be extended. Changes occurring in the skin structure and chemical composition during the fellmongering and tanning processes should be studied, in order that those characteristics that influence leather quality may be determined. At the same time, a survey of the problems of the pelt and leather industries and classification of defects into those caused by inherent skin weaknesses and those resulting from faulty processing would help to define problems of economic importance awaiting investigation.

ACKNOWLEDGEMENTS

I wish to express my gratitude to members of the Ruakura Animal Research Station and the Massey Agricultural College staffs for the help I have received from them during this investigation.

Special thanks are due to Professor A. L. Rae and Dr. C. P. McMeekan; to Dr. W. G. Whittlestone, Mr. N. T. Clare, Miss P. Bartram, Dr. J. James for help and advice, Mr. J. C. D. Hutchinson of C.S.I.R.O., Sheep Biology Laboratory, Prospect.

For technical assistance I am indebted to Mrs. R. Holmberg, Mr. D. Somerville, Mr. J. Kerr, Mr. B. Miller, Mr. J. Gibson, and Mr. B. Wright of Ruakura.

This work was carried out under the tenure of the Shell Scholarship and while a guest member of the staff of the Ruakura Animal Research Station.

NEW ZEALAND
JOURNAL OF SCIENCE

Vol. 1, No. 2, June 1958

CONTENTS

Doppler Measurements on the Soviet Satellites. *A. H. Allen* and *J. E. Drummond*

Sulphur Isotopic Variations in Nature: Part 6—Sulphur Isotopic Measurements on the Discharge from Fumaroles on White Island. *T. A. Rafter, S. H. Wilson,* and *W. B. Shilton*

An Attempt to Extend the Operating Temperature of Copper Cathode Geiger Tubes. *R. E. Belin* and *A. E. Bainbridge*

Coccids Associated with Rata and Kamahi in New Zealand. *J. M. Hoy*

The Spectrophotometric Determination of Molybdenum, Tungsten, and Vanadium in Soils and Plants. Part 1—In Soils. *J. H. Watkinson*

Podocarpus spicatus Heartwood Extractives. *H. V. Brewerton*

Marine Fouling at the Port of Lyttelton. *T. M. Skerman*

Immature Stages of *Eriococcus orariensis* Hoy (Homoptera Coccidae). *J. M. Hoy*

The Heat Pulse Method for Measuring Rate of Sap Flow in a Plant Stem. *R. L. Closs*

Incidence of Coccidia (Sporozoa) in Wild Rabbits *Oryctolagus cuniculus* (L) in Hawke's Bay, New Zealand. *P. C. Bull*

Some Relations to be Expected Between the Directional Spectra of Swell Observed at Different Times and Places on the Ocean. *N. F. Barber*

An Investigation of Some New Zealand Sands for Foundry Moulding. *J. E. Scahill*

CONTENTS

PAGE

Photosensitivity Diseases in New Zealand. XII. Concentration of the Facial Eczema Poison. *E. P. White* 433

Molybdenum in Relation to the Occurrence of Xanthin Calculi in Sheep. *H O. Askew* 447

The Use of Chlorpromazine in the Measurement of Radioactive Iodine in the Thyroid Gland of the Rat. *Evan Wright* 455

A Survey of Collar Rot and Root Rot of Cox's Orange Pippin and Other Apple Varieties in Nelson and Marlborough. *C. E. Woodhead* 462

The Goitrogenic Effect of Thousand-headed Kale on Adult Sheep and Rabbits.. *Evan Wright* and *D. P. Sinclair* 477

Septoria drummondii Ell. et Ev., a Common Seed-borne Pathogen in European Lines of *Phlox drummondii* Hook. Seed Imported to New Zealand. *H. T. Wenham* 486

Non-toxicity to Animals of Ryegrass Endophyte and Other Endophytic Fungi of New Zealand Grasses. *I. J. Cunningham* 489

Techniques of Measuring Pasture Production in Fertiliser Trials. *I. L. Elliott* and *P. B. Lynch* 498

A Quick Method for Applying Insecticide Dust to Experimental Small Animals. *P. L. Thomas* and *B. O'Grady* 522

Damage by Clover Case-bearer Caterpillars. *J. M. Kelsey* 525

Races of Potato Blight, *Phytophthora infestans* (Mont.) de Bary, in New Zealand. *C. M. Driver* 527

The Use of Condensed Whey as a Food for Fattening Pigs. *A. C. Dunkin* 531

The Effect of Light and Temperature on the Chemical Composition of Pasture Plants. *N. O. Bathurst* and *K. J. Mitchell* 540

Molybdenum Deficiency of the Hop. *H. O. Askew, R. J. Monk,* and *Joyce Watson* 553

Studies of Grazing of Crop Forages by Sheep. I. The Effect of Grazing on Composition, Digestibility, and Nutritive Value of Rape. *A. F. Greenall* 569

Studies on the Thickness and Chemical Composition of the Skin of Sheep. *Manika Wodzicka* 582

SUBSCRIPTIONS. **Subscriptions will be accepted only on advance payment and only for a full calendar year. Price: 30s. per annum; single copy 5s.; post free (surface mail). Published in alternate months, starting February. Subscriptions should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 8018, Wellington, New Zealand. Cheques should be made payable to the Department of Scientific and Industrial Research, and if drawn on banks outside Wellington, must have exchange added. Subscribers are requested to give their name and full postal address.**

This journal may also be ordered through The High Commissioner for New Zealand, 415 Strand, London W.C.2, or through any bookseller.

Printed by Coulls Somerville Wilkie Ltd.,
Wellington, New Zealand.

New Zealand Journal of Agricultural Research

VOLUME I No. 5
OCTOBER 1958

21 NOV 195

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind, however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. 4¼ × 7 in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is 4¼ × 7 in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation, etc., of references, see examples in this issue.

NEW ZEALAND JOURNAL of AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. A. Black.

Vol. 1. October 1958 Number 5

EFFECTIVENESS OF HONEY BEES AND BUMBLE BEES IN THE POLLINATION OF MONTGOMERY RED CLOVER

By I. W. Forster* and W. V. Hadfield†

(*Received for publication, 15 May, 1958*)

Summary

In February 1954 and again in February 1955, 35 hives of honey bees were placed immediately adjacent to 10 acres of Montgomery red clover (*Trifolium pratense*) growing in an area considered to be favourable to bumble bees.

Insect activity was noted and it was computed that honey bees provided 77% of the effective pollinating insects in 1954 and 89% in 1955.

Spot checks made at several different points showed this to be a fair cross-section of insect activity on red-clover crops in South Canterbury.

Introduction

Two distinct strains of red clover (*Trifolium pratense*) are in common use in New Zealand: cowgrass or broad red clover and Montgomery red clover.

New Zealand supplies not only her own seed requirements of both clovers but, in most seasons, has a considerable surplus available for export. Montgomery red clover in particular has a ready demand overseas. While high seed yields are quite common from cowgrass, they are not usual in the case of Montgomery red clover, this crop being recognised as a shy seed setter (Hilgendorf). Under very favourable conditions, individual crops have yielded over 400 lb dressed seed per acre, but the average yield for New Zealand, over a 15-year period, is no more than 66 lb per acre. During that period the average annual yield varied from 32 to 155 lb per acre.

As red clover is practically self-sterile, it requires cross pollination before it can set seed. Being an entomophilous plant its flowers are

---

* Horticulture Division, Department of Agriculture, Oamaru.
† Agronomist, Extension Division, Department of Agriculture, Timaru.

N.Z. J. agric. Res. 1: 607–619.

designed for insect pollination and it is therefore dependent on insects for this service. Because it is not an open type of flower, the anthers and stigma are not readily accessible and it would seem that only those insects that continuously gather nectar and pollen, and have the necessary strength to spring the keel of the flower, are of any consequence as pollinators. This rules out the more or less casual visitors to flowers, such as flies and moths, and leaves only honey bees and bumble bees as worthwhile pollinators.

The natural affinity between bumble bees and red clover had long been apparent to observers. The history of agriculture in New Zealand had recorded spectacular increases in red clover seed yields with the introduction of bumble bees in 1839. However, from late in the last century concern had been regularly expressed at the decline in red-clover seed yields, which is reported to have significantly coincided with a marked decrease in the bumble-bee population (Canterbury Agricultural College 1954; Thomson 1922) despite further bumble-bee importations in 1906. There are no definite records, however, to show that this reduction in seed yields and lessening of bumble bees has actually been the steady pattern of events (Gurr, unpublished report).

Honey bees have long been recognised as superior to other insects in the general field of pollination. The permanency of the colony not only allows honey bees to be present in large numbers at any time of the year, but also requires that they seriously and diligently visit flowers for nectar and pollen. Honey bees make full use of their field force as their working methods are highly organised. Though not completely domesticated, they can be concentrated at any particular point as required, because they are amenable to control.

The oft repeated factual assertion that red-clover florets are about 10 mm deep and that the tongue of the honey bee is never more than 7 mm in length, has created a large body of opinion that honey bees cannot work red-clover flowers. This conclusion is unfounded because it is the whole proboscis that is inserted into the flower and, therefore, the reach of the tongue is governed by the distance this organ can be protruded and the amount of thrust that the bee can impart. Obviously actual tongue length does not provide the complete answer (Snodgrass 1925). Also the activities of pollen-gathering honey bees have been largely overlooked, and the fact that the distance down to any nectar would be less than 10 mm according to the quantity present.

Methods used by workers overseas to direct honey bees on to red clover by feeding hives with syrup infused with the aroma of red-clover flowers were applied in New Zealand (Palmer-Jones and Smellie 1950). However, so much skill and labour is necessary for the successful operation of the method that the authors decided that it is doubtful if its practical application has much future in New Zealand.

Casual observations had made it plain that many short-tongued bumble bees (*Bombus terrestris*) adopted the habit of biting through the side of the florets. Although it has been stated that any floret cut at the base in this manner fails to set seed (Canterbury Agricultural College 1954), it is not clear whether it has been proved to what extent such mutilation affects the capacity of the floret to set seed if it has been previously, or is subsequently, pollinated. Obviously the bumble bee piercing the sides of the florets must fail to achieve pollination, and any work done would need to assess to what extent this factor reduces the effectiveness of bumble bees as pollinators.

Observations made from time to time by one of us (I.W.F.) had established the fact that honey bees were regular visitors to red-clover crops in New Zealand. Although red clover is not a dependable source of nectar, red-clover honey has been regularly identified in bee hives and large amounts of red-clover pollen are common. The important role of the honey bee as a pollinator of red clover in other countries was fast receiving recognition and it was considered desirable that some steps should be taken to define the position in New Zealand. The Extension Division, Department of Agriculture, Timaru, had already selected an area for investigations into red-clover seed production and the opportunity was therefore taken to record some aspects of insect activity on this crop. Although it was realised that observations would be intermittent because time was limited, it was felt that much valuable information could be obtained.

EXPERIMENTAL AREA

The experimental area was on the farm of Mr. S. A. Marshall at Taiko, situated approximately 9 miles from Timaru. The countryside is of a rolling formation, dropping sharply for nearly 400 ft into the wide fertile Taiko Valley about half a mile west of the crop under observation. The soil is described as a Claremont silt loam. The district is mainly good clean pasture with a fair amount of cropping and some small-seeds production. Gorse grows in the vicinity in hedges, on roadsides, and in shallow gullies. Several home gardens in the locality contain the usual range of nectar- and pollen-producing plants.

The vast escarpment that forms the eastern side of the Taiko Valley carries considerable growth of mixed vegetation comprising gorse (*Ulex europaeus*), broom (*Sarothamnus scoparius*), cabbage tree (*Cordyline australis*), lawyer (*Rubus*), matagouri (*Discaria toumatou*), five-finger Jack (*Nothopanax arboreum*), kowhai (*Edwardsia microphylla*), koromiko (*Hebe salicifolia*), mahoe (*Melicytis macrophyllus*), broadleaf (*Griselinia littoralis*), putaputawheta (*Carpodetus serratus*), mullein (*Verbascum thapsus*), and tussock.

The area was considered ideal for bumble bees. The terrain and flora offered ample cover for hibernating queens, also nesting places and a reasonable continuity of nectar and pollen supplies.

The crop of Montgomery red clover, about 10 acres in extent, had been sown down in the autumn of 1953. Growth was even and carried abundant bloom during both the 1954 and 1955 flowering season. In all respects the crop appeared an ideal one for seed production.

Thirty-five hives of bees were placed on the edges of the crop when the red clover was just starting to flower. The hives were of medium strength in 1954 and of full strength in 1955.

Owing to the dry conditions in 1954 no other flowers of any consequence were blooming within bee range to compete with the red clover for the attention of insects. In 1955, thistles and a sparse brassica crop were being visited by honey bees and some scattered white clover by both honey bees and *Bombus terrestris*.

EXPERIMENTAL PROCEDURE

Weather Observations

Air temperatures were recorded. Relative humidity was taken with a wet and dry bulb. In 1954 soil temperatures were taken in a hollow and on a ridge. General climatic conditions were noted.

Counts of Bees on Crop

An attempt was made to obtain counts by the method of sweeping with a net used by Palmer-Jones *et al.* (1954), but as it was necessary to record working habits as well as insect species and density, this was abandoned in favour of counting over a set area. A strip 4 ft × 5½ ch was measured out across a representative portion of the field giving an area of 1/30 acre. Counts were obtained by walking slowly down this track and then multiplying the result by 30 to give the number of bees per acre.

It was expected to find all three of the species of bumble bee that had at that time been definitely identified as being present in New Zealand, i.e., *Bombus terrestris*, *B. ruderatus* and *B. subterraneus* subsp. *latreillellus* (Dumbleton 1948). Because *B. ruderatus* and *B. subterraneus* are difficult to differentiate with any certainty under field conditions, and because both have long tongues and are of similar habits, they were grouped together under the name of *B. ruderatus* type.

Additional Counts

In order to obtain a cross section of insect activity over the South Canterbury district, spot counts were made on red-clover crops as opportunity offered during February 1955. Small apiaries had been moved on to some of these crops for pollinating purposes and the average concentration of bee hives would be a little above normal for the district but nowhere near that of the trial crop. On these occasions it was not convenient to work out an area before commencing to count, so a 4-ft stick was used as a guide. Counts were made along this strip for a distance of approximately 5½ ch. The operator then retraced his steps and using the 4-ft stick as a unit, measured the exact length of the strip over which the count had been made. From the figures obtained the density of bees per acre was computed.

Seed Yields

It was planned to use a system designed to compute the actual amount of seed produced per acre. The proposed method was to work out the number of flower heads per acre by making random counts within a wire gauge, 1 ft square. The number of seeds from 100 flower heads picked at random would complete the information required to calculate the total amount of seed in the crop. It was considered desirable to have this theoretical yield as a check on the actual amount of seed obtained by the farmer because it is recognised that a certain amount of seed is lost in the ordinary process of harvesting. This wastage could be a variable factor according to weather, condition of crop and the efficiency of the equipment used.

However, as time was not available to carry out this detailed work the actual quantity of seed harvested was taken as the measure of the performance of each crop, these figures being considered reasonably reliable for the purpose of comparison. Also in a trial of this nature it is very difficult to establish a satisfactory standard for comparison of yields. The only convenient check was a comparison with crops throughout the district, none of which had hives of bees placed on them at anywhere near the concentration that existed on the trial crop.

RESULTS

Counts of Bees on Crops

Honey bees and both long- and short-tongued bumble bees were usually present on the crops although no *B. ruderatus* type were recorded in those counts made in January 1955.

In 1954 the average number of honey bees per acre on the trial crop at Taiko was 1392, pollinating bumble bees 414, and non-pollinating bumble bees (i.e. bumble bees biting the sides of the florets), 765.

In 1955, the figures for the trial crop were: honey bees 1505, pollinating bumble bees 180, and non-pollinating bumble bees 54, while the spot counts on various red-clover crops gave average per-acre densities of 1146 honey bees, 162 pollinating bumble bees, and 86 non-pollinating bumble bees.

Even under optimum conditions, bee counts, including both honey bees and bumble bees, did not quite reach one bee to the square yard. In making bee counts the number of each species working the sides of the florets was recorded, as this information is of utmost importance in weighing their worth as pollinators.

Honey-bee counts showed a greater diurnal variation than did bumble-bee counts (Tables 1 and 2 and Fig. 1), tending to build up during the morning and decrease during the afternoon. Honey bees also showed a greater response to temperature. At 60°F honey-bee activity decreased sharply and in the spot counts, full particulars of which are not shown in table form here, honey-bee activity ceased at 59°F while bumble bees continued working, although mainly in sheltered corners and at a slower rate. The complete absence of bumble bees at 5 p.m. on 7 February 1955, compared with the relatively high honey-bee count, cannot be explained.

In Fig. 2, "effective bumble bees" are those of all species working the tops of the flowers and thus achieving pollination, while "non-effective bumble bees" are the *B. terrestris* biting holes in the sides of the florets and so failing to pollinate. It will be noted in Fig. 2 that

TABLE 1. WEATHER CONDITIONS, INSECT COUNTS PER ACRE AND GENERAL INFORMATION, 1954

Feb. 1954 Date	Time	Weather	Air Temp.	Relative Humidity	*B. terr.* Working Florets from Top	*B. terr.* Working Florets from Side	*B. rud.* and *sub. lat.*	Honey Bees	Remarks
9	2 p.m.	Sunny, light easterly breeze			180	210	—	870	Crop in full flower. Very small nectar loads.
9	4 p.m.	Sunny, calm	68°F.	95%	420	210	90	1590	Insufficient nectar to obtain refractometer readings.
10	2 p.m.	Hot, sunshine, calm	76°F.	66%	300	870	—	3360	Very small nectar loads.
10	4 p.m.	Warm sunshine, calm	72°F.	73%	570	900	630	2850	Crop in full flower. About $\frac{1}{8}$ of flowers seeded. Buds still coming.
11	9 a.m.	Warm, cloudy, N.W. breeze	71°F.	45%	120	1110	120	480	Bumble bees very sluggish. Many completely inactive.
11	11 a.m.	Warm, cloudy, N.W. breeze	75°F.	44%	150	1140	240	1020	Extremely small nectar loads. 2 honey bees working through holes made in sides of florets by B. terr.
12	9 a.m.	Fine, calm. Light cloud	65°F.	70%	60	810	360	930	
12	11 a.m.	Light easterly breeze	69°F.	72%	150	510	120	2310	Very small nectar loads.
15	2.30 p.m.	Warm, some cloud. Has been raining			60	720	270	150	Crop wet.
18	4 p.m.	Misty rain.			—	1170	300	360	

TABLE 2. WEATHER CONDITIONS, INSECT COUNTS PER ACRE AND GENERAL INFORMATION, 1955

Feb. 1955 Date	Time	Weather	Air Temp.	Relative Humidity	*B. terr.* Working Florets from Top	*B. terr.* Working Florets from Side	*B. rud.* and *Sub. lat.*	Honey Bees	Remarks
7	5 p.m.	Cold. S.E. wind			—	—	—	720	Crop in full flower. Very small nectar loads.
8	noon	Cloudy. Has been raining	69° F.	70%	180	30	90	2400	Crop wet. Very small nectar loads.
9	noon	Has been raining	60° F.	83%	60	60	60	60	Bumble bees wet and sluggish. Honey bees prospecting in flowers.
10	9 a.m.	Fine. Sunny. Very light E. breeze	63° F.	74%	150	90	30	600	Very small nectar loads.
10	10 a.m.	Fine. Sunny. Very light E. breeze	66° F.	71%	120	30	60	1380	Very small nectar loads.
10	11 a.m.	Fine. Sunny. Calm	69° F.	63%	60	—	60	2760	Nectar loads a little larger but still small.
10	noon	Fine. Sunny. Calm	73° F.	61%	180	120	30	3180	Nectar loads small.
10	2 p.m.	Fine, sunny. Freshening E. wind	72° F.	61%	90	60	30	1650	Nectar loads small.
10	3 p.m.	Fine, sunny. Very light E. wind	71° F.	68%	150	60	90	2010	Nectar loads very small.
10	4 p.m.	Fine, sunny. Light E. breeze	70° F.	64%	180	60	180	1440	Nectar loads very small.
10	5 p.m.	Fine, sunny. Light E. breeze	70° F.	64%	90	90	90	360	Crop in full flower. Nectar loads extremely small

bumble bees were more numerous in 1954 than in 1955 but the pollination value of the greater concentration in 1954 was largely offset by the greater percentage working through the sides of the florets. *B. ruderatus* type did supply a slightly higher percentage of pollinators than *B. terrestris* in 1954 (Table 3) but only half as many in 1955, while on the spot counts the species were about equal. Table 3, while separating those insects working the sides of the florets from the effective

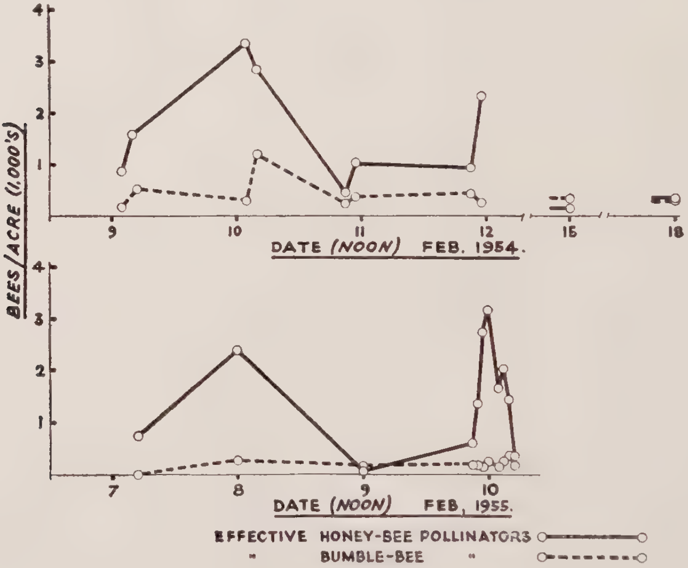


Fig. 1.—Counts of honey bee and bumble bee pollinators.

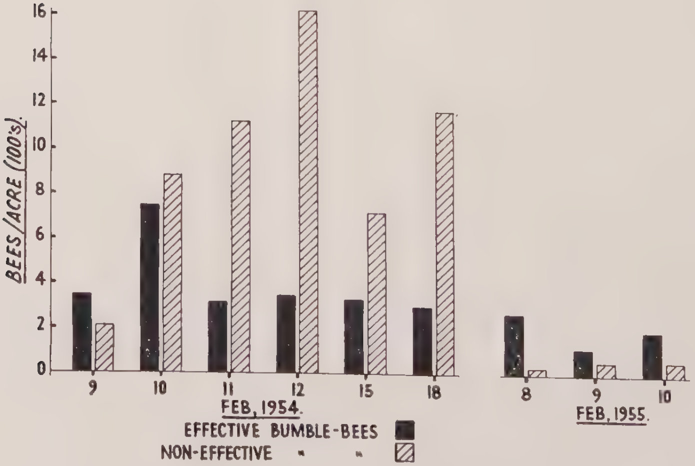


Fig. 2.—Average daily counts of pollinating bumble bees and non-pollinating bumble bees.

pollinators, does include all insects. This means that the large number of *B. terrestris* working the sides of the florets in 1954 gives a high total count, and thus the 53.9% of honey bees, as shown in Table 3, is no indication of the proportion of pollination performed by them. The vital comparison is graphically depicted in Fig. 1 which summarises the relative number of pollinators supplied by honey bees as compared with bumble bees.

TABLE 3. PERCENTAGES OF INSECTS OF VARIOUS SPECIES AND WORKING HABITS

Year	Location	*B. terr.* Working Florets from Top	*B. terr.* Working Florets from Side	*B. rud.* and *sub. lat.*	Honey Bees Working Florets from Top	Honey Bees Florets Working from Side
1954	Trial area at Taiko	7.7%	29.7%	8%	53.9%	0.2%
1955	Trial area at Taiko	6.5%	3.1%	3.7%	86.5%	—
1955	Spot counts over various crops	5.8%	6.3%	5.7%	82.2%	—

Bee Behaviour

Honey bees and bumble bees working from the tops of the florets were gathering pollen. Very little nectar was being gathered by honey bees as the honey sacs of the many bees examined were nearly empty. This was also borne out by examination of the experimental hives which showed that red-cover pollen was being stored but no new nectar was being brought in.

On the trial plot at Taiko, *B. ruderatus* were confined mainly to the end of the counting strip nearest to the gorse hedge and on the spot counts showed a noticeable preference for sheltered locations.

While the two honey bees working the holes bitten by the *B. terrestris* (Table 3), made a rambling approach to the flower, the *B. terrestris* themselves were quite consistent in their working methods. Those working the sides of the florets went straight to that part of the flower and the others went straight to the top.

Speed of Working

An assessment was made of the speed of working of the different species of bee by counting the number of florets visited per minute. While honey bees worked at a fairly consistent rate, varying only between 20 and 16 florets per minute, bumble bees were not so consistent and visits varied from 31 florets per minute to complete in-activity when rain or low temperatures had reduced the bumble bees to a state of immobility. No attempt was made to decide at what stage of low activity bumble bees should be discounted and the counts shown include all bumble bees present on the crop.

While any assessment of the overall working speeds of honey bees as opposed to bumble bees must be an arbitrary one, it would appear that on the counts as made, each species should be credited with a like average speed of working.

Sugar Content of Nectar

An endeavour was made to record the sugar content of the red-clover nectar by removing the honey sacs of bees caught on the crop and placing the contents on the prism of a pocket refractometer graded to give sucrose as a percentage. However, nectar loads were so small that the contents of from 6 to 12 honey sacs were required to give a reading, and as the material thus tested was probably honey brought from the hive the results cannot be taken as having any significance.

Soil Temperatures

No significant relationship was apparent between soil temperatures and insect activity.

Seed Yields

In 1954, 240 lb of machine-dressed seed per acre was harvested from the trial crop at Taiko. Although this was double the average for the district, taken over approximately 80 crops, it was exceeded by the yield from eight other crops which produced from 243 to 400 lb per acre.

The harvest from the trial crop in 1955 was 274 lb of machine-dressed seed per acre, being exceeded by 12 other crops in the district with yields of from 277 to 480 lb per acre. The seed yields from the Taiko crop were the highest the farmer had ever experienced.

DISCUSSION

Although observations were not continuous, the insect activity and results recorded at Taiko over the two red-clover flowering seasons, together with the spot checks on other red-clover crops, give a fair cross-section of conditions throughout South Canterbury.

A very significant feature of the trials was the large number of *B. terrestris* biting holes in the sides of the florets and so greatly reducing the value of the bumble-bee population. However, *B. terrestris* actually provided a greater number of effective pollinators than did the *B. ruderatus* type, and, providing the mutilation of the flowers by side-biting individuals does not directly affect the flowers' ability to set seed, *B. terrestris* appears to be of at least equal importance to *B. ruderatus* in the pollination of Montgomery red clover. Only two honey bees were observed working through holes bitten by the bumble bees and even they did not act as though confirmed in this habit.

Practically the entire field force of honey bees may thus be regarded as effective pollinators.

The smaller number of bumble bees in 1955 could have been the result of various undetermined factors. The only recorded condition which may have caused this fluctation was the greater amount of competing flora present in 1955, which by dispersing the relatively small bumble-bee population, would reduce the number present on any particular area. Honey bees, on the other hand, would be numerous enough to saturate the competing sources without reducing the numbers on any particular crop. It is interesting to note (Fig. 2) that, except for the high count on 10 February 1954, the effective bumble bee population remained fairly constant. Any increase in bumble-bee numbers seemed to be within the non-effective "robbing" group. This could mean that it is greater competition for nectar that drives *B. terrestris* to bite the sides of the florets. This competition would be mainly between bumble bees, as honey bees in this trial gathered mainly pollen and therefore did not affect the supply of nectar in the flowers. *B. terrestris* biting the sides of the florets gather no pollen and so nectar must be the only attraction to them. It was noticeable that both honey bees and *B. terrestris* working the tops of the florets, because of their shorter tongues, had to force the keel of the flower much further apart to reach into it than did *B. ruderatus*. For this reason they may be more deliberate pollinators.

In assessing the number of effective pollinating insects, it is necessary to discount those that work the sides of the floret. On this basis we find that honey bees provided 76% of the pollinators at Taiko in 1954, 89% at Taiko in 1955, and 87% over scattered crops in 1955. The close agreement of these figures supports the contention that this is a fair indication of the worth of honey bees in red-clover pollination.

Although 1954 and 1955 were excellent for the production of red clover, the yields of 240 and 274 lb per acre on the trial area can be considered quite satisfactory, even when compared with the high district average of 120 lb per acre in 1954. It is also interesting to consider that Sweden which is often held up as the ideal for red-clover seed production, produced an average of 251 lb per acre during the 10-year period 1921-31 (Canterbury Agricultural College 1954). As this Swedish average probably includes "cowgrass" types which set seed much more freely, the figure could be considerably higher than the yields for comparable late-flowering types.

In the absence of observations made during the flowering season, it is not possible to determine just what factors operated in favour of those South-Canterbury crops that produced yields in excess of the Taiko trial plot. The entire district is fairly heavily stocked with apiaries, so those crops could easily have as heavy a concentration of honey bees as the trial area. It is interesting to note that the average number of honey bees per acre working the trial crop with its heavy concentration of bee hives was only 15% more than that of the bees working the various crops where hive concentration was comparatively light. In fact the maximum count on the trial area of less than one bee per square yard appeared to be only a fraction of that which the hives on the crop were capable of supplying.

Although it was not possible to record a definite comparison, the honey-bee field force in 1955 did appear to work the red clover more keenly than in 1954, despite some competition from other flora that did not exist in the previous year. This could be the result of the stronger condition of the colonies placed on the crop in 1955. The increased amount of seed produced in 1955 could well be a reflection of this circumstance when it is considered that normally the yield tends to decrease in the second consecutive year of harvesting for seed.

In 1955, when the hives were all at one end of the crop, an endeavour was made to ascertain the effect on seed yield of proximity to the hives. Seed was harvested separately from 10 plots, each 1-yard square, situated at intervals of 1 chain from the apiary. The figures obtained showed no significant trend. The only variation was that caused by differences in the growth of the crop.

CONCLUSIONS

South Canterbury is a district where conditions are favourable for bumble bees yet these studies would indicate that honey bees play the major part in pollinating red clover, and the maintenance of a dependable honey-bee population is definitely in the interests of red-clover seed growers.

It is essential to have honey-bee colonies at full strength for pollination purposes.

The effectiveness of the available bumble-bee population is greatly reduced by the large numbers of *B. terrestris* that bite through the sides of the florets. In spite of the side-biting habit of some individuals, *B. terrestris* is probably of equal value to *B. ruderatus* as a pollinator of red clover because of its superior numbers.

While the deliberate stocking of crops at a rate of 3½ hives of honey bees per acre did not give outstandingly high seed yields, these were well above average and compared favourably with those reported from other countries. As seed set did not decrease at distances up to 10 chains from the hive, it would appear that scattering the hives through the crop is not necessary in the case of small or moderate sized fields.

Further work is necessary to decide to what extent crops can be beneficially and economically stocked with honey bees.

ACKNOWLEDGEMENTS

Thanks are due to Mr. S. A. Marshall for his co-operation in making his crop available for this experiment and to Davidsons' Apiaries, Timaru, for the hire of bees and the placing of them where and when required.

REFERENCES

CANTERBURY AGRICULTURAL COLLEGE 1945: Red Clover and Bumble Bees. *Agric. Bull. 190, Christchurch, N.Z.*

DUMBLETON, L. J. 1948: Bumble Bee Species in New Zealand. *N.Z. J. Sci. Tech. A29:* 308.

HILGENDORF, F. W. [N.D.] "Pasture Plants and Pastures of New Zealand", 4th ed., p. 61. Whitcombe & Tombs, N.Z.

PALMER-JONES, T.; BARTRUM, F. A.; FORSTER, I. W.; HARRISON, D. L. 1954: Effect on Honey Bees of D.D.T. plus Superphosphate Applied as a Dust to White Clover Pasture. *N.Z. J. Sci. Tech. A36:* 177.

PALMER-JONES, T.; SMELLIE, E. 1950: Conditioning of Bees to Control Pollination. *N.Z. J. Agric. 80:* 49.

SNODGRASS, R. E. 1925: Anatomy and Physiology of the Honey Bee," p. 58. McGraw-Hill, New York.

THOMSON, G. A. 1922: "The Naturalisation of Animals and Plants in New Zealand", p. 276. C.U.P.

EFFECT ON HONEY BEES OF SOME DEFOLIANTS

By T. PALMER-JONES* and I. W. FORSTER†

(Received for publication, 11 June 1958)

Summary

Two commonly used commercial defoliants, a mixture of 50% dinitro-ortho-secondary-butyl-phenol (DNBP) plus 10% dinitro-ortho-secondary-amyl-phenol (DNAP) and a 40% solution of penta-chloro-phenol (PCP) were each applied from the ground to four- and ten-acre fields of white-clover pasture shut up for seed. The first defoliant was sprayed at the rate of 2 pints in 10 gallons of dieselene per acre and the second at the rate of 4 lb of 100% penta-chloro-phenol in 10 gallons of dieselene per acre. The clover fields, which were very attractive to bees, were sprayed at a time when bees were visiting them. Owing to the strong repellency of the defoliants no bee mortality occurred.

Plot tests confirmed these results and also showed that the defoliant mixture in aqueous solution and a dieselene spray were strongly repellent to bees.

Laboratory tests proved that sprays of the above defoliants were highly toxic and dieselene moderately toxic to bees.

Plot and laboratory tests showed that the defoliant endothal (disodium, 3, 6-endoxo-hexahydrophthalate) can be applied in safety to plants visited extensively by bees without causing mortality. Endothal does not repel bees.

INTRODUCTION

The production of seed from white and red clover and lucerne is an important industry in New Zealand. Until recently the farmer was often faced with the problem of how to harvest seed satisfactorily from these crops when growth was heavy and the lateness of the season rendered it unlikely that the crop would dry out sufficiently. Now, however, it is possible to obtain pre-harvest drying of these crops after the seed has set by the use of defoliant sprays. The seed can be harvested within a few days of their application.

If it is necessary to spray lucerne and red clover with defoliants, the time of application usually falls in April when these crops are unattractive to bees.

Particularly in seasons which favour luxuriant growth, it is often necessary to defoliate white clover in February at a time when the crop has still many flowers which are attractive to honey bees. The two principal defoliants used at present on white clover are a mixture of dinitro-ortho-secondary-butyl-phenol plus dinitro-ortho-secondary-amyl-phenol and penta-chloro-phenol. Dinitro-ortho-secondary-butyl-phenol is known to be toxic to honey bees and penta-chloro-phenol is a commercial insecticide likely to be dangerous to bees.

It was considered possible that the use of these defoliants on clover pasture attractive to bees might cause heavy mortality. Accordingly

---

* Animal Research Station, Department of Agriculture, Wallaceville.
† Horticulture Division, Department of Agriculture, Oamaru.

N.Z. J. agric. Res. 1: 620–626.

the following experiment was carried out to discover what effect they would have on honey bees when applied to clover pasture containing a high percentage of flowers.

Another defoliant is endothal, disodium, 3,6-endoxo-hexahydrophthalate with the empirical formula $C_8H_8O_5Na_2$. Its toxicity to honey bees had not been investigated so plot and laboratory tests were undertaken.

EXPERIMENTAL

Area

The two ten-acre experimental fields were situated close together on the farm of Mr. A. V. Inch at Highbank near Methven in the South Island. A heavy growth of white clover (*Trifolium repens*) covered both fields which had been shut up for seed. The pastures were thick with clover flowers which were very attractive to bees. A few clumps of red clover (*Trifolium pratense*) and some thistles were scattered through the clover pastures.

Because of heavy rains the growth of clover was so luxuriant that the farmer could not have harvested the seed satisfactorily without the use of defoliants.

Thirteen commercial apiaries were present in a circle of two miles radius with the experimental fields as its centre: three of these apiaries were about a mile distant and one, of fifteen strong hives, was only 300 yd away. There was thus ample bee coverage.

Application of Sprays

Both the defoliants were commercial preparations, one being a mixture of 50% dinitro-ortho-secondary-butyl-phenol and 10% dinitro-ortho-secondary-amyl-phenol, and the other a 40% solution of pentachloro-phenol. The first of these will hereafter be referred to as DNBP plus DNAP and the second as PCP.

The defoliants were applied as in normal practice from a standard low-level boom spray rig fitted to a tractor. The DNBP plus DNAP was applied at the rate of 2 pints in 10 gallons of dieselene per acre and the PCP at the rate or 4 lb of 100% PCP in 10 gallons of dieselene per acre.

Counts of Field Bees

Counts of field bees in the experimental areas were made as described by Palmer-Jones *et al.* (1957). The total bee count obtained for 400 sq. yd was multiplied by 12.1 to give the bee densities per acre plotted in Figs. 1 and 2.

Counts of Clover Flowers

The procedure followed is that described by Palmer-Jones *et al.* (1958) except that counts were made at intervals of five paces instead of ten.

The following counts were obtained on 20 February:

Field sprayed with PCP—

Seeded clover heads	314
Flowering clover heads	262
Total clover heads per 50 sq. ft	576

Field sprayed with DNBP plus DNAP—

Seeded clover heads	295
Flowering clover heads	408
Total clover heads per 50 sq. ft	703

Results

Weather Conditions

Weather was suitable for maximum bee activity on the 20th and the morning of 21 February but was suitable for only limited activity on the afternoon of the 21st. On the 22nd, weather conditions favoured maximum bee activity early in the morning but were cold and wet in the afternoon. Weather was unfavourable for bee activity on the morning of the 23rd but improved greatly in the afternoon.

Counts of Field Bees

Field Treated with DNBP plus DNAP. Fig. 1 shows bee densities on the field prior to 23 February. The density for the 23rd was based on a bee count from a neighbouring field. This was more sheltered from a N.W. wind which blew in the afternoon than the sprayed field and so would give a somewhat higher bee count. The spray was applied by 1 p.m. on the 23rd to the central four acres of the field. The other six acres had been sprayed on the 21st when there was no bee activity.

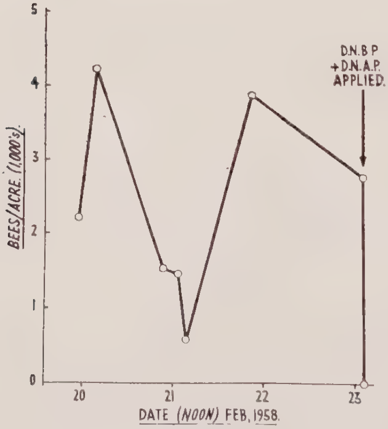


Fig. 1.—Before DNBP + DNAP spray.

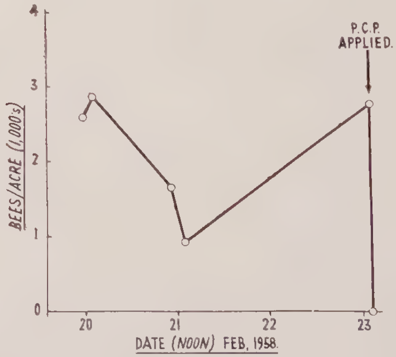


Fig. 2.—Before PCP spray.

Field Treated with PCP. Peak bee densities were less in this field than in the other because it was more exposed (Fig. 2). Before a count could be made on the 22nd, a storm developed and all bee activity ceased for the rest of the day. Bee density on the 23rd is taken as before from the control field. Application of spray took place between 2 and 3 p.m. on the 23rd.

Effect of Defoliants upon Field Bees

Both sprays acted as repellents and practically no bees visited sprayed clover flowers. But, in spite of the very strong smell of the sprays, bees visited odd unsprayed flowers even when these were surrounded by acres of sprayed pasture. Thistles which were sufficiently high to escape the spray remained very attractive to bees.

Effect of Defoliants on Clover Flowers

Because defoliants act more effectively at high temperatures, it is usual to apply them in hot weather. The weather was not very hot at the time of the experimental applications and so the defoliants would take longer to wither up the clover flowers than usual. However, from the time of spraying until withering some two hours later the clover flowers remained unattractive to bees. Under conditions more favourable for application flowers would wither in about an hour.

Bumble Bees

Only two bumble bees were observed on the experimental fields. These were either *B. ruderatus* or *B. subterraneus* sub-sp. *latreillellus,* it not being possible to distinguish between these species in the field.

Plot Tests at Oamaru

Three separate plots at Oamaru were each treated with different defoliants:—(i) DNBP plus DNAP in dieselene, (ii) PCP in dieselene, (iii) dieselene alone. DNBP plus DNAP and PCP were applied at the same rates as in the large-scale experiment and dieselene at the rate of 10 gallons per acre. Each defoliant was sprayed over 30 sq. yd of clover pasture, the treated areas being about 200 yd from an apiary of sixteen hives. The plots were sprayed at 2.30 p.m. on 26 February when weather conditions were suitable for moderate bee activity and there were 629 bees per acre on the field.

No bees visited the plots treated with DNBP plus DNAP nor the one to which PCP had been applied while only a few were seen on the plot sprayed with dieselene.

The flowers in the plot sprayed with dieselene were affected very little in one hour, but those in the other plots commenced to wither in about forty-five minutes. It was apparent from this and later observations that dieselene alone was not nearly so effective as when mixed with either of the two defoliants.

Plot Tests at Wallaceville

Late in March, tests were carried out at Wallaceville on 15 sq. yd plots of pennyroyal plants (*Mentha pulegium*), clover flowers not being available. Weather conditions were suitable for maximum bee activity at the time of the tests and the pennyroyal was attracting many bees.

DNBP plus DNAP in dieselene, PCP in dieselene, and dieselene alone, were applied at the same rates as in the plot tests at Oamaru. DNBP plus DNAP was also applied at the rate of 2 pints in 10 gallons of water per acre.

At the time the defoliants were applied to the plots, honey bees averaged 8 per square yard on the pennyroyal. In all cases the plots became repellent to bees immediately after spraying and remained so until the flowers wilted.

Endothal

This is a water-miscible defoliant unsuitable for application in dieselene. In commercial practice it would be applied at the rate of 1 to 2 gallons in 40 to 50 gallons of water per acre.

Endothal was applied in water to plots with an area of 15 sq. yd at the maximum rate of 2 gallons in 40 gallons of water per acre. The endothal spray repelled bees for only a few minutes after which they returned in the same numbers as before. Bees picked off flowers recently sprayed with endothal and kept under observation in cages supplied with sugar syrup showed no undue mortality.

Laboratory Tests

Because defoliants are more effective at high temperatures, they are usually applied in the hottest part of the day when conditions also favour bee activity. Although it has been shown that the defoliants tested would not cause bee mortality after application, some losses might occur during spraying. So a method developed by Palmer-Jones (1958) was used to study the spray effect of the defoliants on bees. Four batches, each of 50 anaesthetised bees, were sprayed with the defoliant under test. "Heavy spray" means that each of the four batches of 50 bees was sprayed with 1 ml of the defoliant and "light spray" means that each batch received under 0.25 ml. Defoliants were sprayed in the concentrations used in previous tests.

TABLE 1. EFFECT OF DEFOLIANT SPRAYS ON HONEY BEES

Defoliant	% Mortality in 24 hr in Four Batches each of 50 Bees		Remarks
	Light Spray	Heavy Spray	
DNBP plus DNAP in dieselene	100	100	Light spray killed in 30 min. Heavy spray killed at once
DNBP plus DNAP in water	100		All dead or dying in 1½ hours
Dieselene	6 average	100	
PCP in dieselene	100		All dead in 4 hours
Endothal	0	8 average	

The "light spray" would approximate more closely than the "heavy spray" to the quantity of spray which might reach a bee in practice and so it is apparent from the table that endothal spray would cause negligible and dieselene moderate bee mortality while sprays of the other defoliants are highly lethal.

DISCUSSION

Peak bee densities were approximately 4,000 bees per acre on the field sprayed with DNBP plus DNAP and 3,000 bees per acre on the field sprayed with PCP. Figures of 2,000 and 3,000 bees per acre on clover pasture were recorded in the Hastings area by Palmer-Jones *et al.* (1958). The number of clover heads in flower on the Hastings fields averaged about 380,000 per acre compared with 292,000 per acre in the fields used in this experiment which had, however, better bee coverage than in Hastings. So it appears that the fields at Highbank, typical of many others sprayed with defoliants this February in Canterbury, were as attractive to bees as average clover pasture. The laboratory tests showed that sprays of DNBP plus DNAP and PCP were highly toxic to bees and there is little doubt that these defoliants would have caused serious bee mortality but for their repellency.

In spite of its complete lack of repellency, plot and laboratory tests showed that endothal is safe to use.

Defoliants are usually applied from tractors which would drive off many bees from the pasture before sprays from the booms could reach them. Aerial application of the defoliants tested is unlikely but if resorted to could not kill more than the maximum number of foragers per acre of crop, say 4,000, before repellency took effect. Not very many acres at a time are treated with defoliants and the bees on the treated pasture would usually come from several apiaries. When it is considered that a single strong hive may have as many as 20,000 field bees (Grout 1949) it is apparent that losses from aerial spraying of the defoliants tested would not be serious.

CONCLUSIONS

The defoliants DNBP plus DNAP in dieselene, PCP in dieselene, and endothal may be applied to clover pasture when honey bees are collecting nectar extensively from its flowers without causing bee mortality.

ACKNOWLEDGEMENTS

Mr. L. A. M. Griffin, Apiary Instructor, Christchurch, first drew our attention to the widespread use in Canterbury of defoliants on white-clover seed crops which still carried sufficient flowers to render them very attractive to honey bees. His fears that the practice might result in heavy losses of bees led to the carrying out of this experiment. We much appreciated the co-operation of Mr. A. V. Inch of Highbank, near Methven, on whose farm the main experiments were carried out.

We are grateful for help received from Mr. W. R. Lobb, Assistant Fields Superintendent, Christchurch; Mr. C. F. Norton, Spraying Officer, N.Z. Farmers' Co-op. of Canterbury Ltd., Christchurch; and Messrs. J. G. Richards and R. K. Darwin, Instructors in Agriculture, and Mr. G. L. Jeffery, Instructor in Apiculture, all of Oamaru.

Mr. A. L. Bryant, Photographer, Wallaceville, drew the figures. A sample of endothal and information concerning it was supplied by Messrs. Ivon Watkins Ltd., New Plymouth. Messrs. J. H. Barbour and Son Ltd., Wellington, gave us a sample of PCP.

REFERENCES

GROUT, ROY A. 1949: The Hive and the Honey Bee. Dadant and Sons, Hamilton, Illinois, U.S.A.: 150.

PALMER-JONES, T.; FORSTER, I. W.; GRIFFIN, L. A. M. 1957: Effect on Honey Bees of Metasystox Applied from the Air as a Spray to Chou Moellier. *N.Z. J. Sci. Tech. A38:* 752.

PALMER-JONES, T. 1958: Laboratory Methods for Measuring the Toxicity of Pesticides to Honey Bees. *N.Z. J. agric. Res. 1:* 290.

PALMER-JONES, T.; FORSTER, I. W.; LINE, L. J. S. 1958: Effect on Honey Bees of Toxaphene and Strobane Applied to White Clover. Pasture. *Ibid. 1:* 694.

EFFECT ON HONEY BEES OF DDT APPLIED FROM THE AIR AS A SPRAY TO LUCERNE; NOTES ON LUCERNE POLLINATION

By T. Palmer-Jones* and I. W. Forster†

(*Received for publication, 26 May 1958*)

Summary

Ten acres of a lucerne crop at Kurow were sprayed from the air with a DDT emulsion ("Stantox") at the rate of 2 lb of 100% pp′-DDT in 10 gallons of water per acre. Prior to spraying, 23 hives of bees had been established on the crop. The spray was applied early in the morning when there was practically no bee activity, but shortly afterwards conditions were suitable for maximum bee activity. No adverse effect on adult bees or brood was observed. The crop was strongly repellent to bees for a day after spraying.

The DDT spray can be used without harming honey bees or bumble bees provided it is applied when they are not on the lucerne. This insecticide is a logical choice for controlling the variety of insects which attack lucerne.

Honey bee and bumble bee counts were made on the crop. All bumble bees were *B. terrestris* which is a more efficient pollinator of lucerne than the honey bee. But, even if allowance is made for this extra efficiency, bee coverage of the crop falls well below that recommended in the U.S.A.

Various weeds in the vicinity of the lucerne competed for the attention of the bees; if these weeds had been destroyed more bees would have visited the crop.

Introduction

Lucerne (*Medicago sativa*) is grown extensively in New Zealand, 93,958 acres being planted for hay or silage in 1954-55 (N.Z. Official Year Book 1957). In addition many acres of lucerne are grown for grazing. Consequently large areas are set aside each year for seed production. It is not intended to review the literature on lucerne pollination but it has recently been shown in the U.S.A. that it is sound commercial practice to increase seed production by moving hives of honey bees into lucerne. As many as six hives per acre, scattered through the crop, may be used, depending on the number of bees drawn off by competing plants (Pedersen *et al.* 1955; Vansell 1951). Lucerne is not particularly attractive to bees and it has been the experience in the U.S.A. that they can be forced to pollinate the flowers effectively only by saturating the crop. The beekeeper must be paid a pollination fee for his services, as under such conditions of overstocking with hives the quantity of nectar collected is negligible.

In New Zealand, the more progressive lucerne seed growers have found it a worthwhile commercial proposition to hire hives so that honey bees are available to reinforce pollinating insects which may be already present.

---

* Animal Research Station, Department of Agriculture, Wallaceville.
† Horticulture Division, Department of Agriculture, Oamaru.

N.Z. J. agric. Res. 1: 627–632.

During the period in New Zealand when bee activity on lucerne is most necessary for pollination, the crop can be severely damaged by infestation with various types of insect. According to Dr. J. S. Timlin of the Entomology Division, Department of Scientific and Industrial Research, Nelson, the main pests of lucerne are the corn ear-worm *Heliothis armigera*, the looper caterpillar of the winter moth *Hybernia indocilis*, the sucking bugs *Eurystylus australis* and *Dictyotus coenosus*, and the apple leaf roller *Tortrix postvittana*. The clover seed chalcid *Bruchophagus gibbus* is present but would appear to be a minor pest of lucerne in New Zealand.

The lucerne seed grower must take prompt steps to protect his crop against such insects by the application of pesticides. But if he uses substances toxic to honey bees while the crop is in flower he will deprive himself of their service as pollinators when they are most needed. Although the use of DDT for the control of these insects has not been investigated in New Zealand, this insecticide is a logical choice for such a variety of pests. It is also cheap, readily available, persistent, and relatively safe to handle. Previously, Palmer-Jones *et al.* (1954) had shown that DDT dusted over clover pasture caused only negligible mortality of honey bees but it is considered that it would act more effectively against pests if applied to the lucerne as a spray.

The following experiment was designed to find what effect DDT would have upon honey bees when applied as a spray to lucerne in flower.

Experimental Area

This was situated on the farm of Mr. D. L. Matheson, Roseneath, on Lake Waitaki, 7 miles from Kurow.

The lucerne crop, approximately 11 acres in extent, was isolated by surrounding hills from other areas of lucerne on the farm and it was considered that very few bees would leave its vicinity.

Twenty-three strong three-storied hives of bees had been established in groups on the crop which was shut up for seed (Fig. 1).

Round the edges of the lucerne were patches of nodding thistle (*Carduus nutans*), Scotch thistle (*Cirsium lanceolatum*), Californian thistle (*Cirsium arvense*), catsear (*Hypochaeris radicata*), viper's bugloss (*Echium vulgare*) and white clover (*Trifolium repens*). All these were in flower and so competing against the lucerne for visits from pollen- and nectar-gathering bees.

Experimental Procedure

Application of Spray

DDT emulsion was diluted with water to give 2 lb of 100% pp′-DDT per 10 gallons. This was then applied from the air at the rate of 10 gallons per acre to ten acres of the crop from an average height of 10 feet. Application finished at 8.15 a.m., on 12 February. Conditions were perfect with a very slight breeze. A few honey bees and bumble bees were on the crop when spraying commenced.

Counts of Field Bees

Counts of field bees were made in a representative area of the crop. Each of us walked slowly along a separate 100-yard track in the crop (Fig. 1) counting honey bees and bumble bees in a zone 1.5 ft. wide on each side. The total area thus covered by both of us in walking to the end of the tracks and back was 400 square yards. The bee count for this when multiplied by 12.1 gave the density of bees per acre (Fig. 2). The method differs from that described previously by Palmer-Jones *et al.* (1957) in which two of us traversed different parts of the crop each time. However, the modification was necessary to avoid damage to the lucerne plants before harvesting.

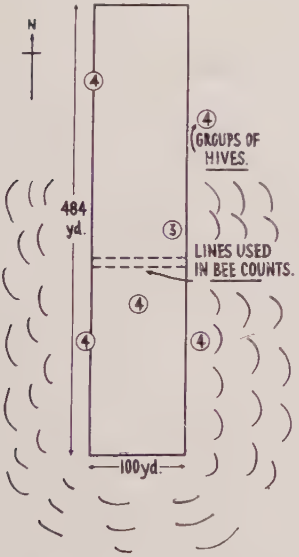


Fig. 1

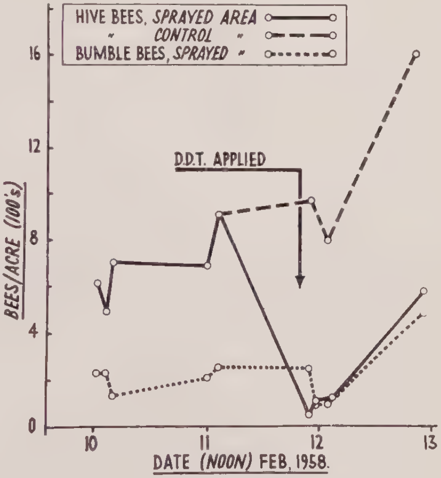


Fig. 2

Fig. 1.—Plan of lucerne crop.
Fig. 2.—Bees per acre on lucerne crop.

RESULTS

Counts of Field Bees

Bee counts were commenced on 10 February and continued through the 11th, the DDT spray being applied by 8.15 a.m. on the 12th (Fig. 2). Weather conditions were excellent throughout this period and suitable for maximum bee activity. Bee counts per acre varied from 500 to 900 prior to spraying but on the day the spray was applied ranged from 50 to 120.

A field of lucerne at a similar stage of growth and with the same honey-bee coverage was used as a control. It was about a mile distant from the treated field from which it was isolated by hills. Bee counts on this field on the day of spraying gave a density per acre of 800 to 970 bees. No mortality of bees was observed on the treated field and so it was apparent that the reduction in numbers was caused by the DDT acting as a repellent.

On the day after spraying, a very high bee density of over 1600 per acre was obtained on the control field. A count on the treated field gave a density of 580 bees per acre but this was made shortly after the other count and, during the interval, the temperature had dropped considerably and bees were leaving the crop. Before long all bee activity ceased for the day. It was considered that a bee count comparable with that on the control field would have been obtained but for the sudden deterioration in the weather. The repellency was thus of only one day's duration.

Heavy rain fell on the night of the 14th and at intervals for several days afterwards so observations ceased.

Counts of Bumble Bees

These were counted in the same way as honey bees. It is apparent from Fig. 2 that bumble bees also were repelled by the DDT only on the day of spraying. It is interesting to note that the first count made at 10 a.m. on the day of spraying was as high as usual but counts dropped afterwards. The probable explanation of this is that bumble bees are less sensitive to cold than honey bees and were already on the crop in fairly large numbers when spraying commenced. In such circumstances there would be a time lag before the spray exerted repellency. On the day following spraying, bumble bees reached a peak of over 480 per acre showing that the DDT had caused little if any mortality amongst them.

Effect of DDT upon Field Bees

The toxic action of the DDT was studied by picking field bees off the crop and placing them in cages provided with feeders holding sugar syrup. These cages were kept under observation in an empty storey above a hive.

On the day the DDT was applied, 8 bees were collected from the crop in the morning and 11 in the afternoon. Only one of these bees died in 48 hours, showing that the spray on the crop was not toxic to bees even immediately after application.

Behaviour of Bees on Crop

Although the crop smelt strongly of DDT on the day of application, the few bees that visited its flowers behaved normally.

Effect of DDT upon Colonies

It will be seen from Fig. 1 that two groups of hives were actually within the sprayed area. These groups were examined during the course of the experiment and later. No adverse effect on adult bees or brood was observed.

DISCUSSION

Under conditions favouring bee activity the average concentration of honey bees on the experimental field may be taken as 850 per acre, equivalent to one per 6 square yards; the average concentration of bumble bees may be taken as 240 per acre, equivalent to one per 20 square yards. The figure of 850 honey bees per acre is obtained by averaging bee densities on the experimental area except on the day of spraying and the following one when they are taken instead from the control field. The figure for bumble bees per acre is derived similarly except that bumble bee densities are taken from the control field only on the day of spraying.

Many observers have drawn attention to the fact that lucerne flowers must be tripped for effective pollination. All the bumble bees observed on the experimental and other lucerne crops at Kurow were *B. terrestris*. This species has been reported as invariably tripping the lucerne flower even when collecting nectar (Gurr 1955). Hadfield and Calder (1936) also reported *B. terrestris* as a very effective lucerne pollinator. Although pollen-collecting honey bees are effective trippers, most of the field bees are usually nectar gatherers which rarely trip the flowers. So it is apparent that one *B. terrestris* is equivalent to several honey bees as a lucerne pollinator.

In the U.S.A., where honey bees alone are generally relied on for lucerne pollination, it is considered that at least 5 per square yard are required (Pedersen *et al.* 1955). Hive bee concentration at Kurow was 0.2 per square yard and bumble bee concentration 0.05. Even if generous allowance is made for the extra efficiency of the bumble bees as pollinators, bee coverage still remains well below that recommended and often attained in the U.S.A. Some increase in bee coverage could have been obtained by eliminating competing nectar sources. The optimum number of hives required per acre for seed lucerne in New Zealand has yet to be determined by trials, and may be different from that of the U.S.A.

We have observed very high concentrations of *B. terrestris*, reaching one per square yard, on clovers in the Lake Tekapo region. There is no doubt that the species plays an important part as a pollinator here and in similar hill country areas in New Zealand. However, bumble bee colonies, unlike those of hive bees, are very weak in the spring, are vulnerable to adverse weather conditions, and cannot be built up in numbers as required. It is therefore unwise to rely solely on them for pollination even in areas where conditions particularly favour their multiplication.

In the coastal areas and plains of New Zealand the reduction of nesting sites through intensive cultivation, mortality through the use of insecticides and lack of spring sources of nectar and pollen, due to the use of weedkillers, will tend to reduce further the already meagre bumble bee population. In these regions effective pollination is dependent on the honey bee.

Palmer-Jones *et al.* (1954) showed that DDT dust repelled honey bees from white-clover pasture for three days. Repellency in this case, although strong, was of only one day's duration and would not affect pollination of the lucerne. An ordinary commercial DDT emulsion called "Stantox" was used in the trial. It cannot be said that "Stantox" has any special properties as a repellent. If it does exhibit more repellency than that caused by the contained DDT this may be due to its formulation as an emulsion.

CONCLUSIONS

It is considered that the DDT spray would not harm hive bees and bumble bees if applied when they were not on the lucerne. The spray is a logical choice for controlling the variety of pests which attack lucerne.

ACKNOWLEDGEMENTS

The authors are grateful for the help of Mr. D. L. Matheson, Roseneath, on whose farm near Kurow the experiment was carried out. We are indebted to Messrs. J. G. Richards and R. K. Darwin, Instructors in Agriculture, and Mr. G. L. Jeffery, Instructor in Apiculture, all of Oamaru; Mr. J. M. Hoy, Entomologist-in-Charge, Entomology Division, Department of Scientific and Industrial Research, Palmerston North; and Mr. R. Odinot, Therapeutant Specialist, Horticulture Division, Department of Agriculture, Wellington. Mr. A. L. Bryant, Photographer, Wallaceville Animal Research Station, drew the figures.

REFERENCES

GURR, L. 1955: A Note on the Efficiency of *Bombus terrestris* (L) as a Pollinator of Lucerne (*Medicago sativa* L.) *N.Z. J. Sci. Tech. A37:* 300.

HADFIELD, J. W.; CALDER, R. A. 1936: Lucerne (*Medicago sativa*). Investigations Relative to Pollination and Seed Production in New Zealand. *Ibid.* 17: 577.

NEW ZEALAND OFFICIAL YEAR BOOK 1957: 62nd issue: p. 250.

PALMER-JONES, T.; BARTRUM, F. A.; FORSTER, I. W.; HARRISON, D. L. 1954: Effect on Honey Bees of DDT Plus Superphosphate Applied as a Dust to White Clover Pasture. *N.Z. J. Sci. Tech. A36:* 177.

PALMER-JONES, T.; FORSTER, I. W.; GRIFFIN, L. A. M. 1957: Effect on Honey Bees of Metasystox Applied from the Air as a Spray to Chou Moellier. *Ibid. A38:* 752.

PEDERSEN, MARION W.; MCALLISTER, D. R.; LIEBERMAN, F. V.; KNOWLTON, G. F.; BOHART, G. E.; NYE, W. P.; LEVIN, M. D. 1955: Growing Alfalfa for Seed. *Utah agric. Exp. Sta. Circ. 135.*

VANSELL, G. H. 1951: Use of Honey Bees in Alfalfa Seed Production. *U.S. Dep. Agric. Circ. 876.*

SPECIFIC GRAVITY AS AN INDEX OF THE FAT CONTENT OF MUTTON CARCASSES AND VARIOUS JOINTS

By A. H. KIRTON and R. A. BARTON, Massey Agricultural College, Palmerston North

(*Received for publication, 3 June 1958*)

Summary

The specific gravities (S.G.) of 58 Romney ewe mutton carcasses have been correlated with total carcass fat content and regression equations computed. A curvilinear regression of the form: Y (per cent fat in the carcass) = 8371.3 S.G. — 4382.9 S.G.^{2} — 3932.9 was fitted to the data. Correlation coefficients and regression equations for S.G. of the 9–10–11 rib-cut, loin, and leg and state of fatness of these joints are presented.

Some factors affecting the accuracy of determining S.G. and the predictive value of S.G. as an index of fat content of carcasses and certain joints are considered.

INTRODUCTION

The density of carcass fat is considerably less than that of other carcass components; hence the larger the proportion of fat, the lower will be the carcass specific gravity (S.G.). In view of this relationship, carcass and body S.G. have been used as an index of the fat content of carcasses of the guinea pig (Rathbun and Pace 1945), the albino rat (Da Costa and Clayton 1950), the pig (Brown *et al.* 1951), cattle (Kraybill *et al.* 1952), the lamb (Stouffer 1955), and the ewe (Barton and Kirton 1956). Correlation coefficients of 0.97 (guinea pig), 0.79 (albino rat), 0.75 (pig), 0.96 (cattle), 0.66 (lamb) and 0.88 (ewe) have been reported by the above mentioned workers. In some cases a straight line and in others, for theoretical reasons (Morales *et al.* 1945), a hyperbola was plotted to fit the data.

MATERIALS AND METHODS

Fifty-eight aged Romney ewes were used in this investigation. Their live weights ranged from 64.2 lb to 203.2 lb and their hot carcass weights ranged from 27.6 lb to 129.7 lb. Forty-eight of the ewes were involved in a study of the effects of *l*-thyroxine and a low plane of nutrition on carcass composition and, in addition, 5 very fat and 5 lean animals were also analysed for fat and other components.

Specific gravity values were determined, on carcasses* which had been chilled for approximately 18 hr. post-slaughter, by weighing to the nearest 0.1 lb in air and to the nearest gramme under water. The water temperature varied from 17°c to 20°c while the surface temperature of the carcasses was 11°c to 12°c. The specific gravity of the thawed-out leg, loin, and 9–10–11 rib cut from the left side of the carcass was also determined and then these joints were dissected into their physical components. The joints were removed from the carcass and dissected following the procedure outlined by Kirton and Barton (1958).

---

* Carcasses were headless and the bones of the extremities including the metacarpals and metatarsals were removed. Kidneys were removed but perirenal fat was left intact.

The methods used to determine the chemical composition of the right side of the carcass and of the 9–10–11 rib cut have already been described by Kirton and Barton (1958). A single ash correction factor and Soxhlet correction factor was applied to the data from the 48 carcasses (c.f. Kirton and Barton 1958) but individual carcass determinations of ash and fat correction factors were carried out on the samples from the series of 5 very fat and 5 lean carcasses.

RESULTS

The S.G. means and the composition of the carcasses and joints studied are presented in Table 1. One of the lean carcasses with a S.G. of 0.997 was very oedematous and accordingly measurements on this carcass were rejected. The remaining 4 lean carcasses, while frozen, lost (shrinkage) on average 6.4% of their cold carcass weight during the 293-day period between determining S.G. and when they were chemically analysed. During the same period the 5 very fat carcasses lost only 1.6% of their cold carcass weight. If it is reasonable to assume that all of the weight lost during cold storage was moisture, then it is possible to calculate the fat percentage as at the time of determining S.G. The calculation indicates that the fat percentages were between 0.4 and 1.4 less than the figures determined on the frozen

TABLE 1. SPECIFIC GRAVITIES, FAT AND WATER PERCENTAGES
MEANS AND RANGES

Item	Number	Mean	Range
Carcass S.G.	48	1.029	1.009— 1.054
Rib cut S.G.	43*	1.013	0.991— 1.039
Leg S.G.	48	1.049	1.030— 1.076
Loin S.G.	45*	1.007	0.984— 1.035
Half carcass chemical fat %	48	40.4	24.9 —54.3
Rib cut chemical fat %	48	50.9	33.4 —68.3
Rib cut dissected fat %	48	51.0	31.4 —69.5
Leg dissected fat %	48	25.4	16.2 —34.7
Loin dissected fat %	48	43.7	25.3 —62.2
Half carcass water %	48	42.2	31.8 —52.7
Carcass S.G.	5	0.996	0.990— 1.004
Half carcass chemical fat %	5	57.8	55.4 —61.7
Carcass S.G.	4**	1.045	1.030— 1.058
Half carcass chemical fat %	4**	19.1	7.1 —34.7

* The method used to determine S.G. of the joints was unsatisfactory early in the investigation and therefore some data were discarded.

** One carcass of this series was discarded because of oedema.

carcasses and used in the correlation and regression equations. In most cases the figure is under 1% but the lean carcasses were slightly more affected than the fat ones.

The correlations between the S.G. of the carcasses and other variates are shown in Table 2 and comparable data on the various joints are given in Table 3. The data shown in these two tables indicate that in most cases S.G. is a good indicator of the fat content of carcasses and joints.

TABLE 2. CORRELATION COEFFICIENTS BETWEEN SPECIFIC GRAVITIES (S.G.) OF THE CARCASSES AND OTHER VARIATES.
(Chemical data unless otherwise stated.)

Other Variate	No. of Pairs	Carcass S.G.	$\frac{1}{\text{Carcass S.G.}}$
Carcass side % fat	57	—0.883	0.880
,, ,, ,, ,,	48	—0.842	0.841
,, ,, ,, ,, (1)	15	—0.826	0.821
,, ,, ,, ,, (2)	15	—0.906	0.906
,, ,, ,, ,, (3)	15	—0.877	0.872
Carcass side % water	48	0.787	—0.785
Rib cut % fat	48	—	0.839
Rib cut % fat (dissected)	48	—0.784	0.781
Rib cut % water	48	0.793	—
Rib cut S.G.	43	0.622	—
Leg fat % (dissected)	47	—0.586	—
Leg S.G.	47	0.395	—
Loin fat % (dissected)	48	—0.731	—
Loin S.G.	44	0.665	—

All correlations significant at the 1% level.
(1) Normal plane: thyroxine treated ewes.
(2) Low plane: thyroxine treated ewes.
(3) Control ewes.
See Kirton and Barton (1958).

TABLE 3. CORRELATIONS BETWEEN JOINT SPECIFIC GRAVITIES AND STATE OF FATNESS OF THE JOINTS

Variates	No. of Pairs	Correlation Coeff.
Rib cut % chemical fat and rib S.G.	43	—0.787
Rib cut % dissected fat and rib S.G.	43	—0.894
Leg % dissected fat and leg S.G.	48	—0.816
Leg % dissected fat and leg $\frac{1}{\text{S.G.}}$	48	+0.816
Loin % dissected fat and loin S.G.	45	—0.795

All correlations significant at the 1% level.

Regression equations based either on S.G. or the reciprocal of S.G. for predicting the fat and water contents of ewe carcasses are shown in Table 4. The standard errors of the regression equations in this table, derived from the reciprocal of S.G., indicate that the theoretically based curvilinear regressions gave no greater prediction accuracy than did the linear regressions.

TABLE 4. REGRESSION EQUATIONS FOR PREDICTING CARCASS FAT AND WATER CONTENT FROM CARCASS SPECIFIC GRAVITY (S.G.)
Dependent variate (% chemical fat in the carcass) = Y
Dependent variate (% water in the carcass) = Z

Population	No. of Pairs	Regression Equation	Standard Error of Estimate
Control ewes	15	$Y = 578.6 - 523.8\ S.G.$	3.12%
Control ewes	15	$Y = \frac{544.1}{S.G.} - 489.3$	3.17%
Normal plane: Implanted ewes	15	$Y = 438.9 - 386.5\ S.G.$	2.88%
Normal plane: Implanted ewes	15	$Y = \frac{408.3}{S.G.} - 355.7$	2.91%
Low plane: Implanted ewes	15	$Y = 534.0 - 479.2\ S.G.$	2.55%
Low plane: Implanted ewes	15	$Y = \frac{496.1}{S.G.} - 441.3$	2.55%
Within above subclasses*	45	$Y = 517.7 - 463.5\ S.G.$	2.85%
Within above subclasses*	45	$Y = \frac{484.0}{S.G.} - 429.8$	2.87%
Total 48 ewes	48	$Y = 537.8 - 483.4\ S.G.$	3.24%
Total 48 ewes	48	$Y = \frac{504.7}{S.G.} - 450.3$	3.25%
Total 48 ewes	48	$Z = 315.9\ S.G. - 282.9$	2.59%

* That is within the Control ewes plus within the Normal plane: Implanted ewes plus within the Low plane: Implanted ewes.

Because the data used to derive the equations in Table 4 did not include a wide range of fat contents, a further series of data from the 5 very fat and 4 lean carcasses were added to the data from the 48 carcasses. A test of significance of departure from linearity (Snedecor 1956, p. 454) applied to these combined data produced a significant result ($p < 0.025$), therefore the equation: $Y = 8371.3S.G. - 4382.9S.G.^2$ 3932.9 fits these data better than a linear equation (where Y = per cent fat in the carcass; standard error of estimate = 4.45 per cent fat, see Fig. 1). This regression line curves in the opposite direction from the theoretically derived curve of Morales *et al.* (1945).

No apparent reason for the departure from the theoretical curve is forthcoming, but the main factor in producing it is the four points marked X in the top half of Fig. 1. These observations were obtained

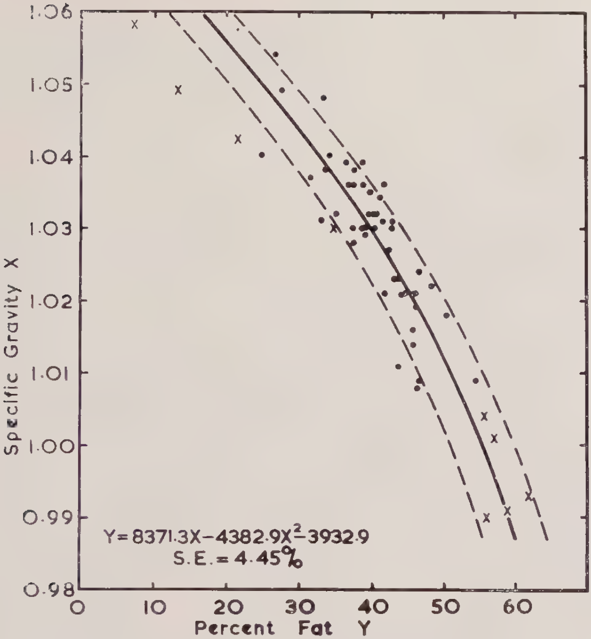


Fig. 1.—Relationship between per cent chemical fat in ewe carcasses and carcass specific gravity (57 observations).

on the four lean carcasses which were not, as far as was known, abnormal in any respect and accordingly were included in the overall regression equation. However, when the data from the carcasses of the 4 lean ewes were omitted, the regression line fitted to the observations from the remaining 53 animals was not significantly curved ($F = 0.88$ for 50 d.f.). The correlation between carcass fat percentage and S.G. was 0.91 and the standard error of estimate of the regression line (per cent fat = 554.1 — 499.2 S.G.) was 3.21 per cent fat.

A least-squares method as used by Beltsville workers (R. L. Hiner 1957, pers. comm.) was employed to estimate from the equations in

TABLE 5. ESTIMATED VALUES FOR SPECIFIC GRAVITY OF FAT AND SPECIFIC GRAVITY OF THE FAT-FREE CARCASS

Population	No. of Pairs	Fat S.G.	Fat-free Carcass S.G.
Control ewes	15	0.9233	1.112
Normal plane: Implanted ewes	15	0.8960	1.148
Low plane: Implanted ewes	15	0.9165	1.124
Within above subclasses	45	0.9136	1.126
Total ewes	48	0.9171	1.121

Table 4 the S.G. of fat and the S.G. of the fat-free carcass. This procedure when applied to the equations based on the reciprocal of carcass S.G., gave the results presented in Table 5. The linear regression equations which were used to estimate these two specific gravities were derived from the theoretical equation proposed by Kraybill *et al.* (1952, p. 579).

The estimated values for S.G. of fat in Table 5 may be compared with a mean value of 0.8999 25°C/25°C (F. B. Shorland, pers. comm.) determined on the perirenal fat of 5 of the 48 carcasses considered in this present paper.

DISCUSSION

The correlations reported here between carcass and joint S.G. and state of fatness are high. In view of these relationships, therefore, it is apparent that carcass fat content can be estimated from carcass S.G. but the curve in Fig. 1 indicates that the regression equations are likely to be more accurate at the higher levels of fatness. However, carcass S.G. is probably not a sufficiently accurate estimator of fat content, because of the size of the standard errors of the regression equations, to be of use when the fat content of an individual carcass is required. Thus with mutton carcasses these relationships are not as high as those reported for the guinea pig (Rathbun and Pace 1945) or for body S.G. and fat content of cattle carcasses (Kraybill *et al.* 1952).

In seeking an explanation for the lower accuracy of S.G. as an index of fat content in ewe carcasses, a number of factors known to influence body and carcass S.G. were considered. For instance, Keys and Brozek (1953) drew attention to the importance of water temperature in S.G. determinations. However, in the present work, water temperature was well within the range of 20°F over which Whiteman *et al.* (1953) state water temperature differences are of no consequence.

The temperature of the carcasses following approximately 18 hr. in the chiller appeared to be reasonably constant. Kline *et al.* (1955) state that a standard chilling time is desirable because variability in this could influence the S.G. of carcasses.

The state of hydration of the body or carcass according to Keys and Brozek (1953) could influence S.G. In this regard, an oedematous condition will in fact lower S.G.; this was apparent in the low S.G. (0.997) of a very lean carcass which had only 2.4% carcass fat. The carcass consisted of 80.9% water on a fat-free basis at the time of determining S.G. Because of the abnormal water content of this carcass, data derived from it were not included in the various statistical relationships presented. The carcasses from those animals treated with thyroxine on the other hand were shown (Kirton and Barton 1958) to be dehydrated to the extent of approximately 1.5% on a fat-free basis. It is possible, therefore, that differences in water content of the carcasses could be one of the factors influencing the accuracy of S.G. determinations and the relationships between S.G. and fat content of some of the carcasses investigated.

The accuracy of fat determinations will be, in part, affected by shrinkage losses occurring during cold storage of the carcasses. Fat percentages were computed from data obtained from carcasses after shrinkage in storage had occurred rather than at the time of determining S.G. This results in a systematic error which should not, however, greatly increase the standard errors of estimate of the regressions.

When the data of Kraybill *et al.* (1952) are compared with those reported here it is clear that their material (cattle carcasses) gave specific gravity figures which were more sensitive indicators of fat content than was the case with ewe carcasses. With their cattle carcasses, a range of 25.9% fat gave a S.G. range of 0.053 whereas with ewe carcasses having a range of 54.6% fat, the S.G. range was only 0.068.

The regression line for the 57 carcasses curves in the opposite direction from the theoretical curve of Morales *et al.* (1945). No apparent reason for this departure from the theoretical curve is forthcoming. It may be noted that Kraybill *et al.* (1952) plotted a very slightly curved hyperbola for their data because of the theoretical considerations of Morales *et al.* (1945). In actual fact when the data of Kraybill *et al.* were tested for departure from linearity a non-significant result ($F = 0.76$ for 27 d.f.) was obtained.

The regression equation for the 15 control carcasses (Table 4) differs from the equation previously reported (Barton and Kirton 1956) because the earlier data were not based on corrected per cent fat; a correction which could not be applied until Soxhlet analyses for the complete series had been carried out. The correction factor when applied to these data had the effect of increasing the estimate of fat in the carcasses by 1–2%.

Data presented in Table 3 indicate that the specific gravities of the joints studied gave a good indication of their state of fatness. As in the case of the carcass as a whole, however, the accuracy was insufficiently high for individual prediction. In view of the known high relationship between state of fatness of the joints and the fatness of the carcass, the correlations (Table 2) between S.G. of the joints and S.G. of the carcass were disappointingly low and of no practical significance.

The determination of S.G. of the joints led to difficulties in addition to those encountered in determining carcass S.G. The cut joint surfaces, especially in the presence of flakey subcutaneous fat as in the loin and 9–10–11 rib cut, allowed a variable amount of air to be entrapped. It was also impossible, under the conditions of the experiment, to standardise the temperature of the joints although water temperature fluctuations were kept at a minimum.

The statistically derived values shown in Table 5 for carcass fat S.G. are in relatively close agreement with actual values determined by Shorland (pers. comm.) working with five perirenal fat samples from these series of carcasses. The S.G. values presented for the fat-free carcass are in reasonably close agreement with the values of 1.098 and 1.094 (Keys and Brozek 1953) for the fat-free body mass of man and guinea pigs respectively. Keys and Brozek reported determinations on the whole body while the present data were based on the carcass; the disparity between the material in these studies will, no doubt, account for most of the observed differences in the S.G. of the fat-free mass.

The usefulness of S.G. as an index of carcass or joint fatness must be considered in relation to alternative measures. Indices of fat content, currently available, which do not involve the destruction of the carcass are dressing percentage (Lush 1926), carcass weight (Barton and Kirton 1958b), and the use of a probe (Hazel and Kline 1952) which can be applied to the live animal or to the carcass.

A comparison of these three methods using the same carcass material has been made (Barton and Kirton 1958a). Carcass weight as an index of carcass fat content was not so subject to errors of measurement as was S.G. or likely to be influenced by seasonal feed conditions as was dressing percentage. Carcass weight as a predictor of fat content, however, will not distinguish between treatment effects which alter, without affecting carcass weight, the proportions of tissues in the carcasses. Dressing percentage (hot carcass weight/live weight) compared well with carcass S.G. despite the fact that significant differences of up to 5 lb of gastrointestinal contents had been induced in some treatment groups. Nevertheless, it would be desirable to know more about the effects of seasonal variations in gut contents and the effects of length of pre-slaughter starvation before dressing percentage as an indicator of carcass fat content can be reliably used. Furthermore, if it is assumed that measurement C (depth of fat over the *longissimus dorsi* m.) is the same or substantially the same fat depth measure as that obtained by using the probe then this technique is unsatisfactory as a means of predicting total carcass fat.

There are limitations to the reliability of S.G. as an indicator of fat content. The method is not sufficiently accurate for individual carcass determinations particularly at the lower levels of fatness. The present work poses the possibility that more factors than have as yet been identified may be influencing the accuracy of the relationship. Support for this view also comes from the studies of Rathbun and Pace (1945) which showed that the experimental curve was shifted by somewhat more than the standard error of the experiment to the left from a theoretical curve and the work of Kraybill *et al.* (1952) in which their curve was shifted to the right of the theoretical curve.

ACKNOWLEDGEMENTS

A financial grant from the Department of Scientific and Industrial Research is gratefully acknowledged. Some aspects of the work were carried out while one of us (A.H.K.) held scholarships provided by the New Zealand Wool Board and the Shell Oil Company.

Finally it is a pleasure to acknowledge the interest and statistical guidance of Professor A. L. Rae throughout this project.

REFERENCES

BARTON, R. A.; KIRTON, A. H. 1956: Determination of Fat in Mutton Carcasses by Measurement of Specific Gravity. *Nature, Lond. 178:* 920.

——————1958a: Assessment of Fat in Mutton and Lamb. *Proc. N.Z. Soc. Anim. Prod. 18:* 112.

——————1958b: Carcass Weight as an Index of Carcass Components with Particular Reference to Fat. *J. agric. Sci. 50:* 331.

BROWN, C. J.; HILLIER, J. C.; WHATLEY, J. A. 1951: Specific Gravity as a Measure of the Fat Content of the Pork Carcass. *J. Anim. Sci. 10:* 97.

DA COSTA, E.; CLAYTON, R. 1950: Studies of Dietary Restriction and Rehabilitation. II. Interrelationships Among the Fat, Water Content and Specific Gravity of the Total Carcass of the Albino Rat. *J. Nutr. 41:* 597.

HAZEL, L. N.; KLINE, E. A. 1952: Mechanical Measurement of Fatness and Carcass Value on Live Hogs. *J. Anim. Sci. 11:* 313.

KEYS, A.; BROZEK, J. 1953: Body Fat in Adult Man. *Physiol. Rev. 33:* 245.

KIRTON, A. H.; BARTON, R. A. 1958: Liveweight Loss and Its Components in Romney Ewes Subjected to *l*-Thyroxine Therapy and a Low Plane of Nutrition. I. Effects on Liveweight, Carcass Weight and Carcass Composition. *J. agric. Sci.* (In press)

KLINE, E. A.; ASHTON, G. C.; KASTELIC, J. 1955: The Effect of Chilling Time on the Specific Gravity of Hog Carcasses and upon the Correlation Between Specific Gravity and Measures of Fatness. *J. Anim. Sci. 14:* 1230.

KRAYBILL, H. F.; BITTER, H. L.; HANKINS, O. G. 1952: Body Composition of Cattle. II. Determination of Fat and Water Content from Measurement of Body Specific Gravity. *J. appl. Physiol. 4:* 575.

LUSH, J. L. 1926: Practical Methods of Estimating the Proportions of Fat and Bone in Cattle Slaughtered in Commercial Packing Plants. *J. agric. Res. 32:* 727.

MORALES, M. F.; RATHBUN, E. N.; SMITH, R. E.; PACE, N. 1945: Studies on Body Composition. II. Theoretical Considerations Regarding the Major Body Tissue Components, with Suggestions for Application to Man. *J. biol. Chem. 158:* 677.

RATHBUN, E. N.; PACE, N. 1945: Studies on Body Composition. I. The Determination of Total Body Fat by Means of the Body Specific Gravity. *J. biol. Chem. 158:* 667.

SNEDECOR, G. W. 1956: "Statistical Methods" 5th ed. Iowa State College Press, Ames, Iowa.

STOUFFER, J. R. 1955: The Effects of High Corn Oil on Lamb Carcass Characteristics and the Use of Specific Gravity as an Index to Degree of Finish. *Proc. 8th Ann. Recip. Meat Conf., Chicago,* p. 87.

WHITEMAN, J. V.; WHATLEY, J. A.; HILLIER, J. C. 1953: A Further Investigation of Specific Gravity as a Measure of Pork Carcass Value. *J. Anim. Sci. 12:* 859.

STUDIES ON THE BIOLOGY OF PEACH RUST (*Tranzschelia pruni-spinosae* Pers.) IN NEW ZEALAND

By HASAN JAFAR, Department of Agriculture, Quetta, West Pakistan
(Colombo Plan Student, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland, New Zealand)

(*Received for publication, 12 June 1958*)

Summary

Peach rust, *Tranzschelia pruni-spinosae,* is of world-wide distribution. It exists in all the fruit-growing districts of New Zealand. In New Zealand, pycniosori and aecidiosori occur only on *Anemone*: uredosori and teleutosori are found on peaches and other stone fruits.

The anemone, in New Zealand, is not important in spreading the disease. The fungus overwinters on twigs of stone fruits as uredosori which produce a new crop of uredospores in the spring.

Almost all the varieties of stone fruits grown in New Zealand are susceptible to rust, though the degree of rust infection varies among varieties.

GEOGRAPHICAL DISTRIBUTION

Peach rust caused by *Tranzschelia pruni-spinosae* Pers. was first described by Persoon (1801). Since then it has been reported from most parts of the world United States of America, Britain, France, Italy, Spain, South Africa, Switzerland, Palestine, China, Japan, Cyprus, Persia, Egypt, Rhodesia, Rumania, Iraq, Portugal, Guatemala, Bulgaria, Turkey, Yugoslavia, Brazil, Ecuador, Chile, Australia, and New Zealand where it was recorded as early as 1897 (Kirk 1897).

ECONOMIC IMPORTANCE

In New Zealand, this rust occurs in all districts where stone fruits are grown. It appears on peaches, plums, apricots, cherries, nectarines, and almonds. It spreads rapidly and assumes epidemic proportions if climatic conditions are favourable for its growth and development during the summer months. It causes premature leaf fall with the result that, in the following season, crop yield is considerably reduced. It also weakens the tree if it persists on the same tree year after year. As infected fruits are blemished and do not keep well, they are unsuitable for the market.

LIFE CYCLE OF THE FUNGUS

Life cycle of the fungus has been described in detail by Cunningham (1925, 1931) and is therefore merely summarised in the present paper.

Tranzschelia pruni-spinosae, being heteroecious in nature, completes its life cycle on two hosts. In New Zealand, uredospores and teleutospores occur on peaches and other stone fruits and pycniospores and aecidiospores on *Anemone coronaria* L.

N.Z. J. agric. Res. 1: 642–651.

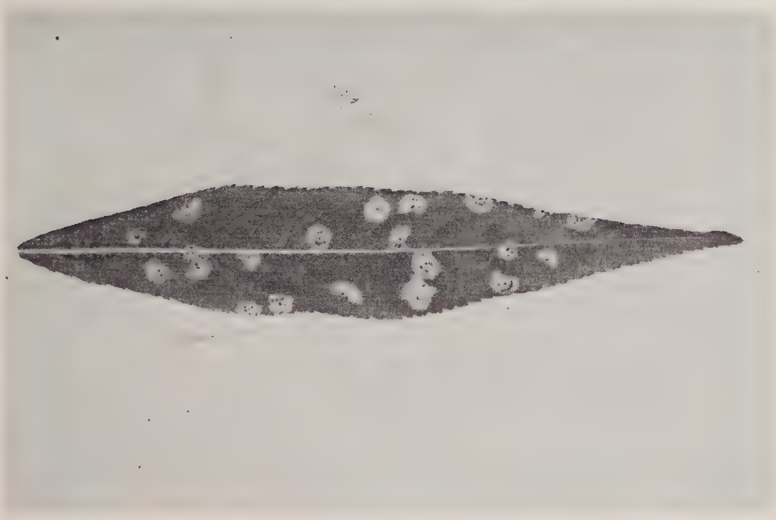


Fig. 1.—Uredosori on the under surface of peach leaf. The sori may be seen as minute black spots scattered in white areas.
× ⅔ Photo: J. W. Endt

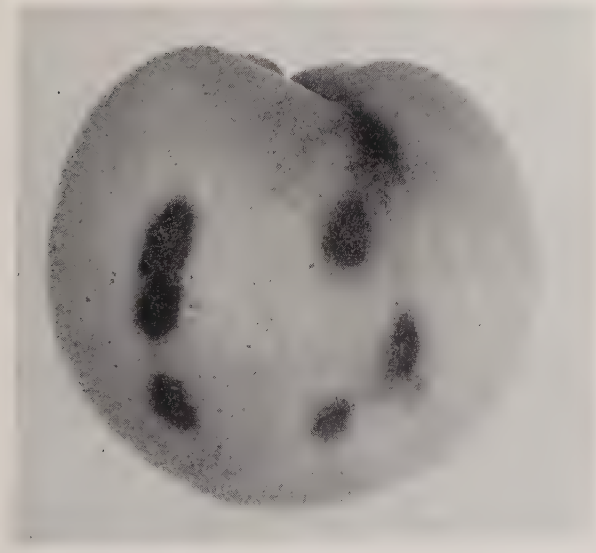


Fig. 2.—Pustules of uredosori on the surface of peach fruit.
× 1 Photo: J. W. Endt

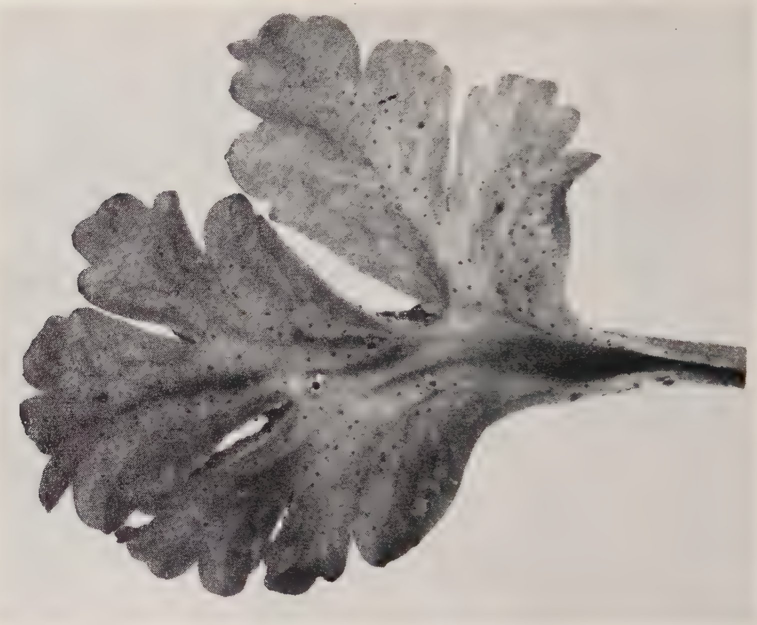


Fig. 3.—Pycniosori and aecidiosori on the upper surface of anemone leaf. Pycniosori are seen as numerous minute black spots scattered on the surface. A few aecidiosori (cluster cups four in number) are also seen.
× 2⅔ Photo: S. A. Rumsey

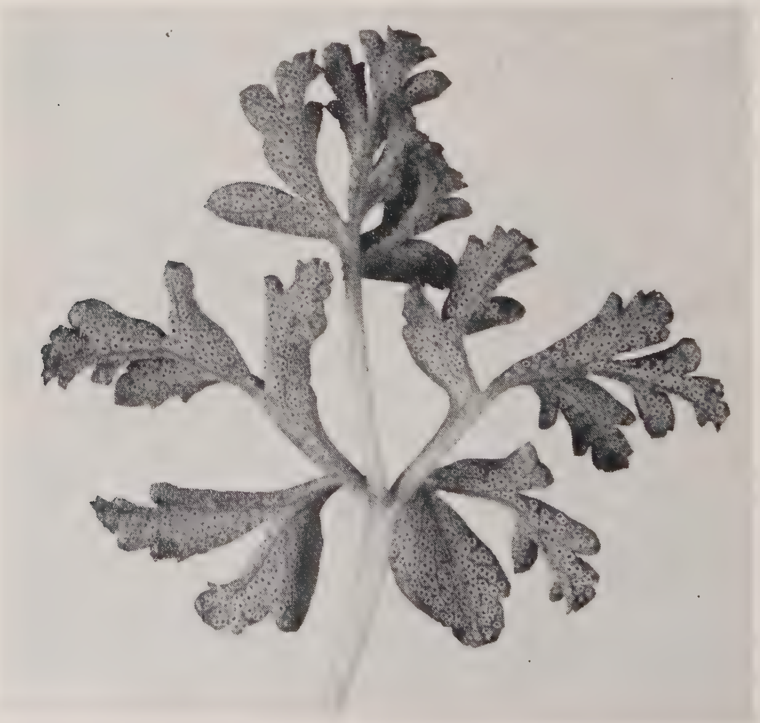


Fig. 4.—Aecidiosori on the under surface of anemone leaf.
× 1½ Photo: S. A. Rumsey

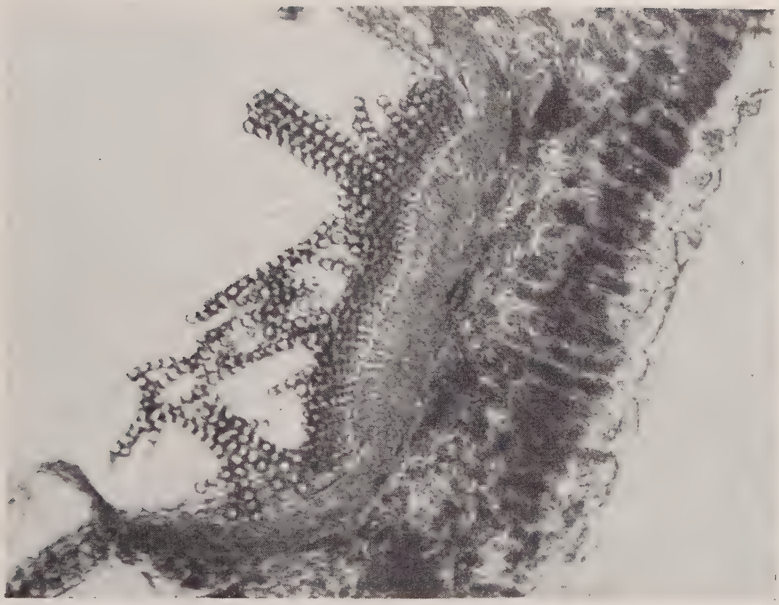


Fig. 5.—A section through aecidiosorus. Note the chains of aecidiospores × 120 Photo: J. W. Endt

Uredospores are produced in pustules or uredosori (Figs. 1 and 2) which are evident from November onward. They germinate readily in the presence of a film of moisture (either dew or rain) and infect stone fruits, but are incapable of infecting anemone. Later in the season teleutospores are produced in the same pustules. Teleutospores, which do not germinate readily, enter a dormancy period. In the following spring when conditions are favourable, they germinate, forming short basidia. Each basidium bears four basidiospores. Basidiospores, on germination, infect anemone but do not infect stone fruits. A few days after infection, pycniosori (Fig. 3) develop. They may occur on either surface of anemone leaves. As a result of sexual process in pycniosori, aecidiosori (Figs. 4 and 5) develop. Aecidiosori may occur on either surface of the leaves but they have a definite tendency to form on the under surface.

The life cycle of the fungus is completed on the infection of leaves by aecidiospores which give rise to the uredosori. Aecidiospores infect stone fruits but are incapable of infecting anemone.

PERPETUATION OF PEACH RUST IN NEW ZEALAND

A study of the life cycle of the fungus reveals that infection on peaches or other stone fruits can be caused either by means of aecidiospores or uredospores. In regions where both anemone and stone fruits are growing, spring infection of the latter by aecidiospores could be important. In New Zealand, however, where the anemone is restricted in distribution, aecidiospores cannot play a major role in infection of stone fruits. As it was known that uredospores could under favourable conditions remain viable for several months, Cunningham (1925) suggested that the fungus was carried over the winter by aecidiospores which lodged in crevices of the bark and bud scales.

Results of a test of viability of uredospores stored for six months at 40°F are given in Table 1. The spores were suspended in water and sprayed on to leaves of potted peach plants. Of 77 inoculated leaves 64 became infected with rust and developed a total of 306 sori.

TABLE 1. INOCULATION OF PEACH PLANTS BY MEANS OF UREDOSPORES STORED FOR 6 MONTHS AT 40°F.

Treatment	Total Leaves at Time of Inoculation	Total Leaves Infected	Total Sori Formed
Inoculated	77	64	306
Check	110	0	0

In view of these facts a search was made to find out the source of overwintering uredospores which provide inoculum in spring. A number of peach trees were examined regularly during the 1957-58 season so that the initial development of infection on the new growth could be observed. It was found that the new leaves with rust pustules were in close proximity to an old lesion or lesions present on bark of twigs (Fig. 6 and Table 2).

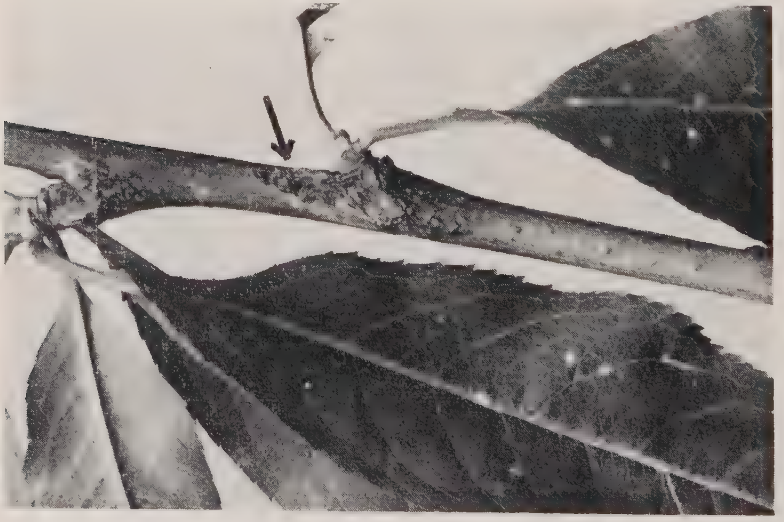


Fig. 6.—Rust infection on new leaves situated in the close proximity of an old lesion on one-year-old twig.
× 1¾ Photo: S. A. Rumsey

TABLE 2. RELATIONSHIP BETWEEN INFECTIONS OF LEAVES AND TWIGS

No. Lesions on Twig	No. Leaves on Twig	No. Leaves Infected
None	30	None
,,	53	,,
,,	28	,,
,,	84	,,
,,	21	1
,,	41	2
1	83	39
1	43	17
1	51	10
1	137	24
2	41	16
2	40	15
2	130	75
3	75	29
3	195	71
4	147	72
4	129	95

In order to make sure that these lesions on the twigs were not the result of fresh infection, a thorough search was made for lesions on current year's growth but none was found. A microscopic study of lesions on one-year-old twigs revealed that they were rust pustules containing numerous uredospores. Pustules on current season's growth were not observed until the end of March when rust was in abundance on peach trees. Pustules on twigs arising from rust infection are

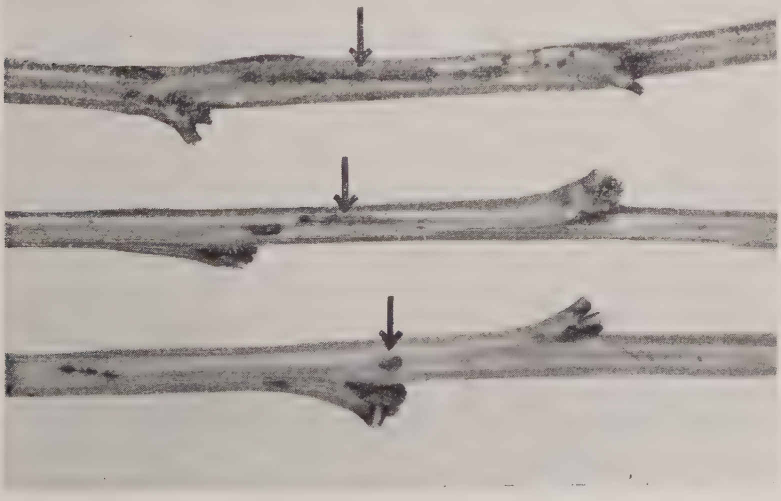


Fig. 7.—Pustules of uredosori on the bark of twigs (current season's growth). Note the longitudinal split in the bark on top twig. × 1 Photo: S. A. Rumsey

brown in colour. They form cankers on the twigs, the fructification causing a longitudinal split in the bark exposing the uredospores (Fig. 7).

The results in Table 2 indicate that the main cause of initial infection during the spring is freshly produced uredospores in old rust pustules on the twigs. The over-wintering uredospores which may be present on the tree do not appear to play a major role in providing the inoculum for infection. The twig infection takes place in the orchards in late summer or early autumn, and the mycelium remains alive during the winter giving rise in the following spring to a new crop of uredospores that infect the leaves. Once the rust spreads to the leaves and new sori are formed, the disease gradually builds up and, by late summer, susceptible trees have become severely infected.

Incubation Period of the Fungus

In order to study the incubation period of the fungus inoculations were made by means of aecidiospores and uredospores.

Spores were suspended in water and sprayed on potted peach plants with an atomiser. Inoculated plants as well as check plants were placed in a humidity chamber (100% R.H.) for 24 hours at 22°c (±1.1°c). The plants were then transferred to a glasshouse. Daily inspections were made for rust infection. Eight days after inoculation, rust pustules were noticed on the leaves of plants sprayed both with aecidiospores and uredospores. Formation of new sori was not observed after ten days.

Incubation period of the fungus is therefore 8–10 days and is the same for both aecidiospores and uredospores.

Varietal Resistance

Varieties of peaches, nectarines, plums, apricots, and cherries available at the Plant Diseases Division were studied for their natural resistance. With the exception of a few varieties where only three trees were available, a sample of five trees per variety was used. From these, 25 leaves per tree were taken at random and the number of sori per leaf estimated. The degree of infection was evaluated by dividing the average number of sori per leaf into grades which proceeded in geometrical progression, i.e. they were equally spaced on the logarithmic scales.

Grades	0	1	2	3	4	5
No. of sori per leaf	0	1	2–3	4–7	8–15	16–31

Grades	6	7	8	9	10
No. of sori per leaf	32–63	64–127	128–255	256–511	512–1023

This system was followed because it had the advantage that more leaves could be sampled in the same time than would have been possible if the numbers of sori per leaf were actually counted, also the analysis of the results was very much simplified.

Peaches

Varieties of peaches studied were: Akarana, Braddock, Briggs Red, Carman, Elberta, Golden Queen, J. H. Hale, Mary's Choice, Mayflower, Paragon, Rosebud, Stark, Triumph.

The varieties appear to fall in four groups (Table 3). Paragon, Elberta and J. H. Hale showed a good degree of resistance, Akarana, Stark, and Triumph were mildly affected. Rosebud, Carman, Mayflower, Briggs Red, Mary's Choice, and Golden Queen appeared to be moderately susceptible, while Braddock was found to be highly susceptible.

TABLE 3. COMPARATIVE SUSCEPTIBILITY OF PEACH VARIETIES TO *Tranzschelia pruni-spinosae*

	A			C	
Variety	Av. Grade Infection/ Leaf	Av. No. Sori/Leaf	Variety	Av. Grade Infection/ Leaf	Av. No. Sori/Leaf
Paragon	0.08	0.06	Rosebud	1.89	2.30
Elberta	0.14	0.10	Carman	2.04	2.70
J. H. Hale	0.74	0.60	Mayflower	3.26	6.70
			Briggs Red	3.50	8.00
	B		Mary's Choice	3.74	9.40
			Golden Queen	3.82	10.00
Akarana	1.15	1.10			
Stark	1.21	1.10			
Triumph	1.27	1.20		D	
			Braddock	9.57	530.00

Sig. diff. 5% 0.79. 1% 1.05.

A survey carried out on the incidence of rust throughout the fruit districts of New Zealand has also shown that almost all varieties of peaches are susceptible to rust. Varieties which showed infection with rust were: American Pound, Blackboy, Buttercup, Carman, Golden King, Golden Queen, Le Vainqueur, Mary's Choice, Millicent, Paragon, Royal George, Stark, and Wiggins.

Nectarines

Hunt's Tawny appeared to be more susceptible than Goldmine on which rust infection was light (Table 4).

As a result of the survey carried out in districts where nectarines are grown, the varieties New Boy and Goldmine were found to be susceptible to rust.

TABLE 4. COMPARATIVE SUSCEPTIBILITY OF NECTARINE VARIETIES TO *Tranzschelia pruni-spinosae*

Variety	Av. Grade Infection/Leaf	Av. No. Sori/Leaf
Goldmine	1.81	2.20
Hunt's Tawny	5.84	40.00

Sig. diff. 5% 3.04. 1% 4.43.

Plums

In the varieties Shiro, Santa Rosa, George Wilson, and Billington, rust infection was mild except on Billington (Table 5).

TABLE 5. COMPARATIVE SUSCEPTIBILITY OF PLUM VARIETIES TO *Tranzschelia pruni-spinosae*

Variety	Av. Grade of Infection/Leaf	Av. No. Sori/Leaf
Shiro	0.23	0.16
Santa Rosa	0.59	0.45
George Wilson	0.83	0.69
Billington	4.72	18.60

Sig. diff. 5% 0.94. 1% 1.31.

Apricots

Rust infection on the varieties Trevatt, Bolton, Moorpark, Newcastle, and Oullin's Early was light and they were not significantly different in the degree of infection, but the variety Roxburgh Red was found to be more susceptible (Table 6).

TABLE 6. COMPARATIVE SUSCEPTIBILITY OF APRICOT VARIETIES TO *Tranzschelia pruni-spinosae*

Variety	Av. Grade Infection/Leaf	Av. No. Sori/Leaf
Trevatt	0.02	0.01
Bolton	0.06	0.04
Moorpark	0.25	0.17
Newcastle	0.42	0.30
Oullin's Early	1.08	0.98
Roxburgh Red	2.86	5.00

Sig. diff. 5% 1.34. 1% 1.95.

Cherries

Occurrence of rust on cherries in New Zealand has been reported (Cunningham 1925). The varieties under observation, viz, Bedford, Chapman, Early Cluster, Florence, St. Margaret, Twyford, Werder's Early, William's Favourite, were free from rust infection in the season 1957-58.

ACKNOWLEDGEMENTS

The writer wishes to express his grateful thanks to Dr. E. E. Chamberlain, Director of Plant Diseases Division, Department of Scientific and Industrial Research, for granting permission to carry out the work at his Division, and to Mr. K. M. Harrow, Senior Principal Scientific Officer of Plant Diseases Division, for instructions and guidance in the investigation.

Thanks are due to Dr. H. R. Thompson of Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for statistical analysis.

Thanks are also tendered to Dr. P. J. Brook of Plant Diseases Division for his suggestions, to Mr. A. T. J. Watts, Stone Fruit Specialist, and to all the Horticultural Instructors of the Department of Agriculture, New Zealand, for supplying specimens and information on the incidence of rust.

The writer is particularly grateful to the Government of New Zealand and the Government of Pakistan for granting a Colombo Plan Award which made this work possible.

REFERENCES

CUNNINGHAM, G. H. 1925: "Fungous Diseases of Fruit Trees in New Zealand". 382 pp. The Brett Printing and Publishing Co. Ltd., Auckland, N.Z.

——— 1931: "The Rust Fungi of New Zealand". 261 pp. John McIndoe, Dunedin, N.Z.

KIRK, T. W. 1897: Plum Rust (*Puccinia pruni*); Peach Curl, Blister Leaf Curl, or Frenching (*Exoascus deformans*); Apple Scab (*Fusicladium dendriticum*), *N.Z. Dep. Agric. 5th annu. Rep.* pp. 133-4.

PERSOON, C. H. 1801: Synopsis methodica fungorum . . . Parts 1, 2, 706.

SOME EFFECTS OF SOIL PHYSICAL CONDITION ON RYEGRASS GROWTH

By D. B. EDMOND, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North.

(Received for publication, 11 April 1958)

Summary

Two experiments are reported, wherein soil physical condition was changed by (1) compaction treatments and (2) compaction and puddling treatments. Seedlings of perennial ryegrass (*Lolium perenne* L.) and short-rotation ryegrass (*L. perenne* L. × *L. multiflorum* Lam.) were used in the winter 1955.

Both ryegrasses were tolerant of increasing soil compaction, the only effects being ephemeral changes in growth form, and differences in root distribution.

Where the soil had been puddled, the growth of both ryegrasses was reduced, but a compaction three weeks after the puddling appeared to remove the worst effects of the puddling.

INTRODUCTION

Compaction of the soil can reduce plant growth (Gupta 1933, Raney *et al.* 1955, Shaw 1952), while Bates (1935) and Shaw reported that puddling of surface soil can have a similar effect. Gradwell (1956) reported a soil puddling without compaction, but it seems likely that both effects will occur together.

Puddled soils form crusts which can restrict diffusion of gases and water (Domby and Kohnke 1956), and in view of Kopecky's (1927) report that, after 24 hours drainage, 5–10% of soil volume should be air space for normal growth of oats, it is clear that puddling may be an important factor affecting pasture growth.

It has been reported (Edmond 1958) that treading can affect pasture growth by influencing plants both directly and indirectly. Thus evaluation of plant tolerance of soil compaction is difficult in pasture where direct influences, such as bruising and crushing, occur. Klecka (1937), and Lieth (1954), classified pasture plants according to treading tolerance, perennial ryegrass (*Lolium perenne* L.) being classed as tolerant.

As part of a study of the effects of animal treading on pasture, it was decided to investigate the effects on ryegrass growth of some modifications of soil physical condition which may occur under normal treading. Seedling plants of New Zealand certified perennial ryegrass and N.Z. certified short-rotation ryegrass (*L. perenne* L. × *L. multiflorum* Lam.) were grown in compacted and puddled soils.

N.Z. J. agric. Res. 1: 652–659.

Experiment 1: Soil Compaction

METHOD

Fertile Manawatu silt loam from the Grasslands Division area at Palmerston North was passed through a $\frac{1}{4}$-in. mesh sieve, dampened and compacted in steel cylinders, 6 in. diam. × 6 in. depth, using an hydraulic press. Pressures of 25, 50, 100, or 200 lb/in.^{2} were applied until compaction appeared to be complete in each case. The effect of these treatments on the soil at the beginning and the end of the trial, is shown in Table 1. One seedling (2 tillers and pruned to $\frac{1}{2}$ in. shoot and $\frac{1}{4}$ in. root) of either perennial or short-rotation ryegrass, was planted in each cylinder (10 May 1955). There were five replications of each species. The cylinders were arranged in sand trays open to the weather, with a permanent water supply $5\frac{1}{2}$ in. below the soil surface.

TABLE 1. VOLUME WEIGHT AND MOISTURE CONTENT OF COMPACTED SOILS

Compaction Pressure lb/in.^{2}	Volume Weight (g/cm^3)				Soil Moisture (g/100 g dry soil)	
	Beginning	End			Beginning	End
	0–6 cm	0–4 cm	4–8 cm	Mean	0–6 cm	0–8 cm
25	1.12	1.61	1.56	1.58	18.6	22.6
50	1.28	1.63	1.61	1.62	19.1	22.4
100	1.41	1.65	1.65	1.65	18.0	21.4
200	1.45	1.69	1.67	1.68	17.0	20.0
S.E. ±	0.03	0.01		0.01	0.4	0.4
Sig. diff. 5% level	0.07	0.02		0.03	1.24	1.19

For the progressive estimation of treatment effects, weekly measurements were made of tiller and leaf numbers, lengths of leaf blade, and, from the fourth week after planting, tiller angles. Tiller angle was taken as being the mean elevation from horizontal of the stiff, basal portion of the tiller. For each tiller, measurements were made of the lengths, widths $\left(\frac{\text{sum of widths } \frac{1}{3} \text{ from each end}}{2}\right)$ thence leaf areas of the leaf blades of the youngest mature leaf (leaf 1) and the next two immature (leaves 2 and 3). Roots were recovered from 0–4, 4–8, and > 8 cm depths. The crowns, which consisted of shoot stubble and interwined roots, were weighed separately. All material was oven dried.

During the 65 days of the experiment, rain fell on 28 days and there were 24 frosts ranging from —0.1° to —7.2°c.

RESULTS

Harvest Measurements

Herbage and whole-plant yield and leaf lengths were not significantly affected by treatment, and are not presented.

Moderate compaction treatments caused significant increases in both species in leaf width, areas, and crown weight (Tables 2 and 3). There were similar increases in root weight due to compaction in the 0–4 cm layer, but below this there was no effect.

TABLE 2. Leaf Measurements of Ryegrass Grown in Compacted Soils

Compaction Pressure lb/in.²	Whole leaf-mean width (mm)		Whole and Short leaves—mean area (mm²)	
	Short rotation	Perennial	Short rotation	Perennial
25	2.19	1.98	95	71
50	2.51	2.36	122	85
100	2.23	2.14	114	91
200	2.28	2.03	98	70
S.E. ±	0.09		8	
Sig. diff. 5% level	0.27		24	

TABLE 3. Dry Weight of Ryegrass Crowns and Roots

Compaction pressure lb/in.²	Crowns (mg)		Roots (mg)			
	Short-rotation	Perennial	Short-rotation		Perennial	
			0–4 cm	4–8 cm	0–4 cm	4–8 cm
25	31.4	39.4	43.6	25.6	36.2	20.2
50	41.4	60.4	53.8	34.0	47.2	20.6
100	46.4	49.0	51.4	28.8	58.8	23.4
200	44.4	55.8	54.0	21.4	56.0	16.6
S.E. ±	4.9		3.3			
Sig. diff. 5% level	13.9		9.3			

Pre-harvest Measurements

Compaction treatments caused significant differences in tiller angle at the 4th and 5th weeks after planting (Table 4). In each case the tillers of both species in less compact soils were more upright, but it should be noted that the measurement was subjective. There were no other significant compaction effects.

Observations

The soil surfaces were puddled by the rain and permeability was restricted. There was some surface heaving during the heavier frosts. Because of the small size of the cylinders the experiment was terminated after 9 weeks growth. At this stage there was no apparent restriction of root growth.

TABLE 4. TILLER ANGLES OF RYEGRASS GROWN IN COMPACTED SOILS

Compaction pressure lb/in.^{2}	Fourth Week after Planting		Fifth Week after Planting	
	Short-rotation	Perennial	Short-rotation	Perennial
25	44°	45°	46°	49°
50	40°	51°	38°	42°
100	34°	36°	33°	43°
200	29°	29°	31°	35°
S.E. ±	4°		4°	
Sig. diff. at 5% level	12°		11°	

DISCUSSION

There were no large treatment effects in the experiment, the most striking change being in habit of growth. As measurements of tiller angle were commenced only after the differences became apparent, it is not known when they developed. After the 5th week of the trial they decreased and disappeared. Leaf widths and crown weights were least in the lightly compacted soil. Yet there was no significant effect on yield. Perhaps yield might have reflected differences in growth habit if the experiment had been protracted or if there had been competition between plants. It is of interest that the widest leaves occurred in the 50 lb/in.^{2} treatment, this pressure being about the same as the hoof load of a cow (Myers 1956).

The distribution of the roots suggested that heavily compacted soil impeded root penetration, as found by Weaver (1919) and others. If this effect persists, surface rooting may become pronounced, thus predisposing the plants to nutrient and water deficiency, and to treading damage.

The effect of the soil crust on permeability was probably small as peripheral cracks were present.

For normal growth of ryegrass, some soil compaction is required (Keen and Cashen 1932). In this experiment, it is possible that the 25 lb/in^2 treatment was sufficient to achieve this. The failure of heavier compaction to affect yield may have been due to the relatively small differences in volume weight which were produced. It appeared that the general tolerance of ryegrass plants to soil compaction suggested by Lieth and Klecka is confirmed.

Experiment 2: Soil Compaction and Puddling

METHOD

An area of the same soil as used in Exp. 1 was rotary-hoed to 4 in. depth in April 1955. Four soil treatments were as follows:

1. Untreated.
2. Soil puddled.

3. Soil puddled and then compacted at 50 lb/in.^{2}
4. Soil compacted at 50 lb/in.^{2}

These were applied in a 2 × 2 × 2 split-plot design, 16 seedlings per plot of both perennial and short-rotation ryegrass being planted at 9 in. centres in 5 replications in early May.

Puddling was effected in mid-April by saturating the soil with water and stirring it vigorously to approximately 3 in. depth with a rake. Three weeks later the appropriate plots were compacted using stilt-like treading equipment, which exerted a pressure of 50 lb/in.^{2} under the operator's weight (Fig. 1).

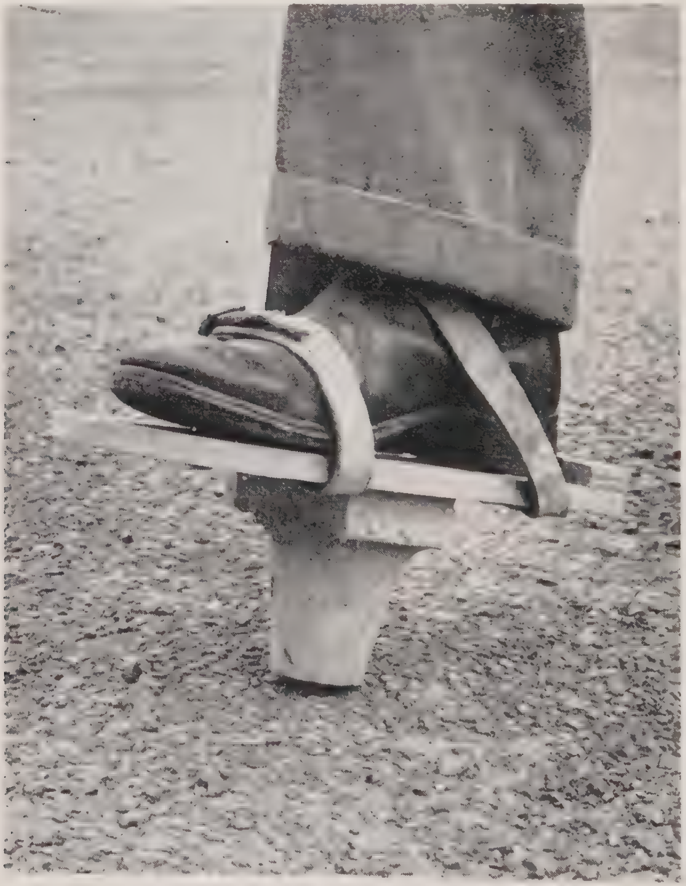


Fig. 1.—Treading equipment to compact soil.

The plants were harvested at ground level on 20 and 21 September 1955. Total herbage from four replicates was weighed green, and oven dried. In the remaining replicate, tiller and leaf numbers and oven-dry weights were determined for all plants. One plant in each plot, selected for approximation to mean yield, was lifted in a soil cylinder 9 in. diam. x 9 in. deep . Roots were recovered from 0–$3\frac{1}{2}$ in., and $3\frac{1}{2}$–9 in. depths and dried.

Near harvest, one soil sample, 1in. diam. × 2 in. deep, was taken from each plot for determination of volume weight and moisture content. Soil resistance to penetration was measured with six measurements per plot.

RESULTS

Herbage Yield

As the ratio of green weight to dry weight was almost constant, only dry weight data are presented (Table 5). Puddling significantly reduced the herbage weight of both perennial and short-rotation ryegrasses, whereas compaction increased it significantly. There was no significant compaction × puddling interaction.

TABLE 5. HERBAGE YIELDS OF RYEGRASS GROWN IN PUDDLED AND COMPACTED SOILS
(g/plot)

	Soil Treatment			
	None	Puddled	Compacted	Puddled and Compacted
Short-rotation	123	90	205	150
Perennial	84	85	160	123
S.E. ±	15			
Sig. diff. at 5% level	44			

TABLE 6. TILLERS, LEAVES, AND DRY WEIGHT OF RYEGRASS HERBAGE
(Per plant, for one replicate)

	Species	Soil Treatment				S.E. ±
		None	Puddled	Compacted	Puddled and Compacted	
Dry Wt. (g)	Short-rotation	5.0	2.2	10.5	8.1	11.9
	Perennial	2.9	3.2	8.0	4.7	
Tiller No.	Short-rotation	55	33	95	81	125
	Perennial	64	57	146	103	
Leaf No.	Short-rotation	201	124	355	281	39
	Perennial	222	215	490	317	

Other Plant Measurements

The herbage-yield data for the one replicate (Table 6) and for the whole experiment (Table 5) were similar. Accordingly for analysis purposes all plot differences in that replicate (Table 6) were assumed to be caused by treatment.

As with herbage yield, soil compaction increased and puddling decreased tiller and leaf numbers. Treatment effects were similar for both species.

There were no significant treatment effects on root yield, but only five plants per treatment were examined, the largest difference being 28% (coefficient of variation 22%).

Soil Measurements

Near harvest and after several rainless days, puddled soil held more water than non-puddled soil (Table 7), while compacted soil held more than non-compacted soil. Both compaction and puddling increased volume weight and resistance to penetration. There was a significant interaction in both these measurements.

TABLE 7. MOISTURE CONTENT, VOLUME WEIGHT AND RESISTANCE TO PENETRATION OF PUDDLED AND COMPACTED SOILS AT END OF EXPERIMENT

		Not Compacted	Compacted	S.E. ±	Sig. diff. at 5% level
Moisture content (g/100 g soil)	Not puddled	15.6	17.4	0.4	1.0
	Puddled	17.8	17.9		
Vol. weight (g/cm^3)	Not puddled	1.25	1.60	0.02	0.07
	Puddled	1.51	1.61		
Resist. to penetration (lb/in^2)	Not puddled	67	260	5	14
	Puddled	149	252		

Observations

Untreated soil was always soft and unstable, but it consolidated as the experiment proceeded. Drainage was impeded in puddled and compacted plots, and as the soil dried the soil surfaces cracked, particularly on the puddled plots. The bluish colouration, typically associated with anaerobic conditions, was seen in all plots save controls, being most marked in those which were both compacted and puddled.

DISCUSSION

In this experiment the puddled soils were compacted, while compacted soils were puddled to some extent during treatment. However, in spite of this the results showed that the soil treatments were different in effect.

The reactions of the two ryegrasses were similar throughout. Soil compaction appeared to favour plant growth, whereas puddling reduced it. In puddled soil gaseous diffusion was probably restricted and the importance of oxygen in soil air is well known. It is possible that an accumulation of carbon dioxide may have affected availability of nutrients (Shaw 1952). The way in which the compaction + puddling treatment was applied, i.e. compaction three weeks after puddling, probably ensured better permeability, from the point of view of plant growth, than in puddled soil.

From both experiments with Manawatu silt loam it appeared that soil compaction was necessary for good ryegrass growth, and that compactions produced by a wide range of pressures differed little in effect. However, puddling of this soil reduced growth. In practice this could be important when wet pastures are trodden. Soil pans which can develop beneath the surface under treading, would probably heighten such effects. The unexpectedly favourable effect of the joint compaction and puddling treatment is tentatively explained in terms of technique. It is possible that treading pasture some time after puddling may promote recovery of vigour.

ACKNOWLEDGEMENTS

The author wishes to thank Mr. E. O. C. Hyde for advice, Mrs. J. Harford and Mr. A. Fryer for assistance, and Miss V. Fleming, Applied Mathematics Laboratory, for statistical analyses.

REFERENCES

BATES, G. H. 1935: *J. Ecol. 23:* 470-87.
DOMBY, C. W.; KOHNKE, H. 1956: *Proc. Soil Sci. Soc. Amer. 20:* 1-5.
EDMOND, D. B. 1958: *N.Z. J. agric. Res. 1:* 319-28.
GRADWELL, M. G. 1956: *Proc. N.Z. Soc. Soil Sci. 2:* 37-9.
GUPTA, P. S. 1933: *J. Ecol. 21:* 452-74.
KEEN, B. A.; CASHEN, G. H. 1932: *J. agric. Sci. 22:* 126-34.
KLECKA, A. 1937: *Sborn Csl. Akad. Zemed. 12:* 715-24. Seen only in *Herb. Abstr. 8:* 822.
KOPECKY, J. 1927: *Proc. 1st. int. Congr. Soil Sci. 1:* 495-503.
LIETH, H. 1954: *Z. Acker-U. PflBau 98:* 453-60.
MYERS, D. J. 1956: *Univ. of N.Z. Thesis,* Six Hoof Features of Dairy Cattle, (Lodged in Massey Agric. College Library).
RANEY, W. A.; EDMINSTER, T. W.; ALLAWAY, W. H. 1955: *Proc. Soil Sci. Soc. Amer. 19:* 423-8.
SHAW, B. T. 1952: "Soil Physical Conditions and Plant Growth." Academic Press, New York.
WEAVER, J. E. 1919: *Carnegie Inst. Publ. 286,* pp. 128.

INVESTIGATION ON THE CONTROL OF PEACH RUST (*Tranzschelia pruni-spinosae* Pers.)

BY HASAN JAFAR, Department of Agriculture, Quetta, West Pakistan (Colombo Plan Student, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland, New Zealand)

(*Received for publication, 16 June 1958*)

Summary

All fungicides tested were effective against peach rust (*Tranzschelia pruni-spinosae*) but zineb, captan and thiram were decidedly superior to lime sulphur plus colloidal sulphur. Zineb appeared to be more effective than captan and thiram although difference in control failed to reach significance at the 5% level.

Soil treatment with potash, phosphorus, and nitrogen fertilisers had no significant effect on incidence of peach rust although the tests suggested that heavy application of nitrogen tended to increase degree of infection.

Spraying of foliage with zinc sulphate plus hydrated lime reduced rust infection on peaches but the reduction failed to reach significance at the 5% level.

Three spray applications during the January-March period following an application in November are likely to keep this rust under control.

INTRODUCTION

Fungicide trials on the control of peach rust caused by *Tranzschelia pruni-spinosae* Pers. have been carried out. In addition to sulphur, which for years has been the standard treatment for rusts, the trial included the newer organic fungicides, captan, thiram, and zineb.

The effects of fertilisers were also tested as potash and phosphate fertilisers are said to have an effect in reducing rust infection (Cunningham 1931).

Bollard (1953) observed that rust infection was severe on potash-deficient peach trees, while potash-treated trees showed very light infection.

Zinc sulphate in combination with hydrated lime was recommended for the control of peach rust in South Africa. (Dippenaar 1943).

EXPERIMENTAL

Effectiveness of Fungicides for Control of Peach Rust

The following materials were used:—

Lime sulphur (15% polysulphide—liquid) + colloidal sulphur 40% paste.
Zineb 65% wettable powder
Thiram 80% wettable powder
Captan 50% wettable powder

There were five single peach tree replications in each treatment including unsprayed checks. Three varieties were included in each

N.Z. J. agric. Res. 1: 660–664.

treatment, two replications being Golden Queen, two Paragon, and one Braddock.

Spraying commenced in the spring at the blossom stage and was continued during the growing season at 21-day intervals until the fruits were harvested (10 applications).

To study the effect of time of spray applications on control of rust an additional treatment with zineb was commenced on the observation of first rust infection in the block. In this treatment the first spray was applied on 20 November 1957 (the day of observation of first rust infection) and continued at 21-day intervals (6 applications).

Infection records were taken at intervals throughout the season by estimating the number of rust pustules per leaf on 25 leaves taken at random from each tree. The number of pustules per leaf were placed in grades which proceeded in geometrical progression.

Grade	0	1	2	3	4	5
No. of sori/leaf	0	1	2–3	4–7	8–15	16–31

Grade	6	7	8	9	10
No. of sori/leaf	32–63	64–127	128–255	256–511	512–1023

Substantial infection did not occur until the first week in March and only the last three readings have been used for the analysis of results. Means of these three readings are given in Table 1.

TABLE 1. MEAN INCIDENCE OF PEACH RUST FOLLOWING DIFFERENT SPRAY APPLICATIONS
(Readings were recorded on 20.3.58, 31.3.58 and 22.4.58)

Treatments	Concentration lb/100 gal. water	Av. Grade of Infection/Leaf	Av. No. Sori/Leaf
Zineb (sprayed after first rust infection appeared) 6 applications	2	0.02	0.01
Zineb 10 applications	2	0.07	0.05
Captan 10 applications	2	0.45	0.32
Thiram 10 applications	2	0.62	0.48
Lime sulphur + colloidal sulphur 10 applications	5 + 1.12	1.78	2.10
Check (no spray)	—	3.96	11.00
Sig. diff. at 5%		0.60	
1%		0.84	

All treatments resulted in a highly significant reduction in the number of rust lesions.

Sulphur reduced the rust infection considerably but it was significantly inferior to the organic fungicides used.

Zineb appeared to be more effective than captan and thiram although the difference in amount of infection failed to reach significance at the 5% level.

Six applications of zineb were found to give as good control as 10 applications.

Fertiliser Trial

This trial was carried out in a block of Golden Queen peach trees planted at 12-ft. spacing on volcanic soil. The fertilisers were applied in a band between 1 and 4 ft. from the trunk of the trees at the time of bud movement and thoroughly incorporated into the soil. Each plot consisted of one tree with a buffer tree on each of its four sides. There were three replications of each treatment, plots being randomised within blocks. The fertilisers tested for their effect on incidence of peach rust were muriate of potash, nitrate of soda, and superphosphate.

A similar system of counting rust lesions and assessing severity of infection was followed as in the fungicide trial. Means of five readings recorded at intervals during January-March are given in Table 2.

TABLE 2. MEAN INCIDENCE OF PEACH RUST ON TREES RECEIVING DIFFERENT FERTILISERS

Treatment	Quantity of Fertiliser lb/tree	Av. Grade of Infection/ Leaf	Av. No. Sori/Leaf
Muriate of potash	3	3.31	7.00
Muriate of potash	12	3.03	5.70
Nitrate of soda	3	2.77	4.70
Nitrate of soda	12	4.70	18.20
Superphosphate	3	2.61	4.10
Superphosphate	12	3.07	5.80
Check (no fertiliser)	—	2.68	4.40
Sig. diff. 5%		2.06	
1%		2.89	

While no significant difference was found among the treatments there was an indication that high nitrogen application tended to increase the incidence of rust.

All trees were making good growth, which suggested that mineral supplies in the soil were adequate and that the additional fertilisers were superfluous. The effect of different treatments on the incidence of rust might have been more pronounced if the soil had been deficient in minerals.

Test with Zinc Sulphate plus Hydrated Lime

A spray with zinc sulphate in combination with hydrated lime reduced incidence of peach rust on Paragon peaches although the reduction failed to reach significance at the 5% level. Average grade of infection per leaf on treated and check trees was 1.36 and 3.15 respectively, the difference required for significance at 5% and 1% being 2.59 and 3.93 respectively.

DISCUSSION AND CONCLUSION

The results of these experiments showed that zineb, captan and thiram effectively reduced incidence of peach rust. None of the fertilisers used in the trial was found to be effective. A spray with zinc sulphate plus hydrated lime gave some reduction of rust infection. However in both the fertiliser and zinc sulphate trials there was much variation in amount of rust infection between trees and no firm conclusion could be drawn about the effect of treatments.

As, in these trials, fungicides were found to give the only promising control, and as time of application seemed to play an important part, a spray schedule can be devised after a close study of mode of perpetuation of rust fungus, time of initial infection, and the progress of rust infection during the season.

In this country, the rust carries over from season to season in bark lesions. It has been found (Jafar 1958) that the twigs of peach trees are infected in early autumn at the time when rust spores are in abundance. The rust overwinters in these twig lesions, which produce a new crop of spores in the spring on the return of suitable climatic conditions. Spores thus produced spread the disease to the leaves. Initial infection takes place in November and the disease gradually develops during the season until, by late summer, infection is general. It would appear that this gradual increase of infection during the season is followed every year. Table 3 shows the pattern in which the disease progressed during the 1957–58 season on five check trees in the spray trial.

TABLE 3. INTENSITY OF RUST INFECTION ON DIFFERENT DATES

Date	25.11.57	17.12.57	13.1.58	4.2.58	10.3.58	31.3.58	22.4.58
No. sori/leaf	0.04	1.10	2.10	1.50	3.10	7.90	51.00

These facts lead to the conclusion that one application with an effective fungicide should be given in November to check the initial infection. Later the trees should be sprayed during January-March at 30-day intervals (3 applications). Four applications should be quite sufficient to keep the rust in check.

ACKNOWLEDGEMENTS

The writer wishes to express his grateful thanks to Dr. E. E. Chamberlain, Director, Plant Diseases Division, Department of Scientific and Industrial Research, New Zealand, for granting permission to work at his Division and to Mr. K. M. Harrow, Senior Principal Scientific Officer of Plant Diseases Division for his guidance in the investigation.

Thanks are due to Dr. H. R. Thompson of the Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for carrying out statistical analysis of the results.

The writer is also grateful to the Governments of New Zealand and Pakistan for granting a Colombo Plan Award which made this work possible.

REFERENCES

BOLLARD, E. G. 1953: Severe Potash Deficiency in Young Peach Trees. *N.Z. J. Sci. Tech. A35:* 39-44.

CUNNINGHAM, G. H. 1931: "Rust Fungi in New Zealand" 261 pp. John McIndoe, Dunedin, N.Z.

DIPPENAAR, B. J. 1943: The Control of Deficiency Diseases in Plants. *Fmg. S. Afr. 18:* 189-94.

JAFAR, H. 1958: Studies on the Biology of Peach Rust (*Tranzschelia pruni-spinosae* Pers.) in New Zealand. *N.Z. J. agric. Res. 1:* 642-51.

SOME LONG-TERM EFFECTS OF FERTILISERS ON A MOWN TURF OF BROWNTOP AND CHEWING'S FESCUE

By D. B. Edmond and S. T. J. Coles*, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 24 April 1958*)

Summary

A study was made of the effects of different fertilisers on a turf of browntop and Chewing's fescue.

Where ammonium sulphate was the predominant fertiliser, a dense uniform turf developed, with increased soil acidity and volume weight, disappearance of earthworms and the formation of a mat of living and dead plant tissue, 1–1¼ in. deep. Mean herbage production was 2,700 ± 360 lb dry weight per acre per annum.

Without the acidifying effect of ammonium sulphate, the turfs were more open and heterogeneous, there was no mat formation, worm activity was high and herbage production reached the estimated level of 9,400 lb dry weight per acre per annum.

Introduction

In 1932, at Palmerston North, the Greens Research Committee of the New Zealand Golf Association began investigation of greenkeeping practices under New Zealand conditions.

As turf composed of browntop (*Agrostis tenuis* Sibth.) and Chewing's fescue (*Festuca rubra* L. var. *commutata* Gaud.) had proved to be suited to greenkeeping practice, it was decided to study different fertiliser treatments applied to this turf, with the object of determining the best treatment for turf maintenance. A description of the investigation and some results have been published by Levy and Madden (1940). Metson and Gibbs (1946) reported soil data secured from the plots in 1941.

This paper describes some soil and plant characteristics studied during the period May 1951 to January 1953, when treatments had been continued for 20 years.

Materials and Methods

A level area of recent alluvial soil (Manawatu silt loam on sand) was prepared and sown in March 1932 with browntop (1 part) and Chewing's fescue (2 parts) at the rate of 300 lb seed per acre. The fertiliser treatments were commenced in April 1932 and continued without change until 1953. Herbage was mown and removed from the plots except for a period in 1943-5. Floods in 1942 and 1953 deposited thin layers of silt over all plots but the second flood occurred after this investigation. For this study, seventeen of the plots were selected,

---

* Present address: C/- Abraham Seed Co. Ltd., Feilding, N.Z.

N.Z. J. agric. Res. 1: 665-674.

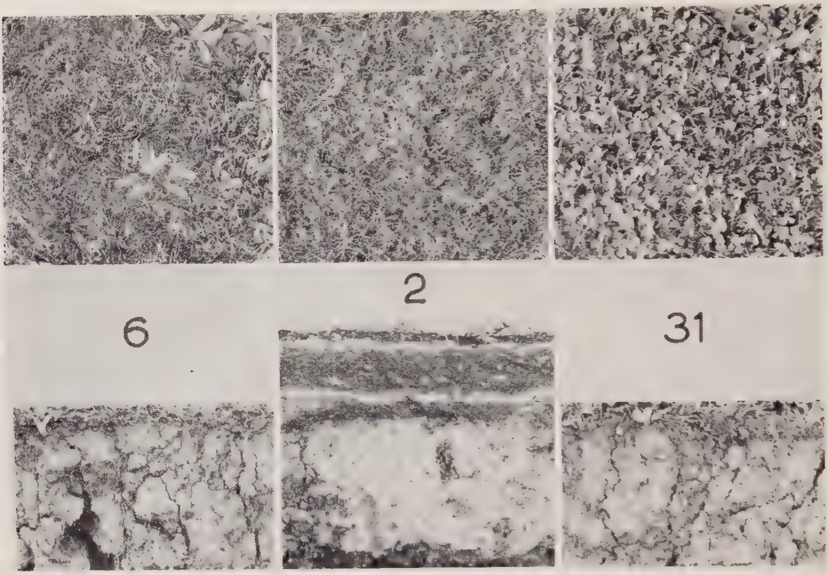


Fig. 1.—Swards and soil profiles of representative turf plots. Plot 6, Not fertilised, no mat. Plot 2, Fertilised, mat. Plot 31, Fertilised, no mat.

their treatments being representative of those used in practice (Table 1). Plots designated "Mat" had developed a mat of dead and partly decomposed plant material on the soil surface (Fig. 1).

The following measurements were made:—

Herbage Cover. Three multilayered point analyses (Levy and Madden 1933) were carried out, using 30 stratified random sets (each of 10 points) per plot.

Herbage Yield. Dry weight was determined each fourteen days, by harvesting a central 25 ft x 2 ft strip at 3/16 in. height. Samples for the determination of dry weight (25–100 g) and soil and worm-cast contamination (20–40 g) were secured at each harvest, the soil and worm casts being separated from the herbage by hand.

Botanical Composition. Botanical dissection was difficult because of the short, narrow leaves, which wilted rapidly. To reduce bias, the samples (5 g green weight) were split between two observers.

Chemical Composition of Herbage. Total ash, insoluble ash, N, P, K, and Ca were determined from bulked samples secured during the late spring–early autumn period, when soil contamination was slight. The methods used were similar to those used by Sears and Newbold (1942).

Worm Counts. Three samples (9 in. square, 18 in. deep) per plot were taken in each of three winters and all worms counted directly.

Soil pH. This was determined using a glass-electrode assembly on 25 samples (0–2 in. deep) per plot, in the winter, spring, and summer.

Soil Resistance to Penetration. Using an hydraulic penetrometer, 25 measurements per plot were taken six times during 1952.

Soil Carbon and Organic Matter Composition. Using the Schollenberger method, carbon was determined on duplicate samples of soil profiles at two sites in Plots 1 and 2. Proximate analyses of soil organic matter were carried out*, using samples from Plots 1 and 2.

Volume Weight and Soil Moisture. Volume weights were determined for various layers at single sites in plots 1, 2, 3, 6, and 8. In winter 1952, moisture content was determined for several layers at two sites in the same five plots.

RESULTS

As the investigation progressed, it was clear that the swards of fertilised "No Mat" plots differed from the weedy slow-growing swards

TABLE 1. PLOTS STUDIED, FERTILISER TREATMENTS (cwt/acre) AND TYPE OF TURF

Plot No.	Am.	P.	K.	Fe.	Ca.	Blood	Nit. S.	Comp.	Turf Type
2	6	—	—	—	—	—	—	—	Mat
3	6	4	—	—	—	—	—	—	
4	6	4	4	—	—	—	—	—	
4A	6	4	4	—	—	—	—	—	
8	6	4	—	2	—	—	—	—	
32	6	4	4	—	—	—	—	—	
32A	6	4	4	—	—	—	—	—	
1	—	4	—	—	—	—	—	—	No Mat
5	6	4	4	—	9	—	—	—	
19	—	—	—	—	—	—	—	35 cu yd	
21	6	4	—	2	—	—	—	35 cu yd	
29	—	4	4	—	—	10.5	—	—	
31	—	4	4	—	—	—	8	—	
6 6A 27 27A					N I L				No Mat

A = plots fertilised each 3 months—all others monthly.

Am.	Ammonium sulphate	20–21% N.
P.	Superphosphate	20–22% P_2O_5
K.	30% potash salts	25–30% K_2O
Fe.	Ferrous sulphate	
Ca.	Ground limestone	45–50% CaO
Blood	Dried blood	10–12% N, 1.0–1.5% P_2O_5
Nit. S.	Sodium nitrate	16.5% N.
Comp.	Compost	Variable

---

* By R. Bailey, Rukuhia Soil Research Station, Hamilton, N.Z.

of unfertilised "No Mat" plots. Since these differences were striking the results have been grouped accordingly (Table 1).

Herbage Cover (Table 2)

"Mat" plots contained few species other than Chewing's fescue and browntop, whereas the others were heterogeneous in composition. Unfertilised "no mat" plots were characterised by less grass, more clover and weeds, and lower turf density than the fertilised "no mat" plots.

TABLE 2. Turf Composition Determined by Multilayered Point Analysis
(Mean no. of hits per 300 points)

	Fertilised		Unfertilised
	Mat	No. Mat	
Browntop	231 ± 19	258 ± 20	173 ± 25
Chewing's fescue	357 ± 16	88 ± 17	98 ± 21
Sweet vernal	Trace *	22 ± 2	9 ± 2
Yorkshire fog	0	11 ± 1	Trace
Poa spp.	8 ± 5	59 ± 5	11 ± 7
White clover	5 ± 4	44 ± 5	18 ± 6
Other clovers	Trace	39 ± 7	101 ± 9
Flat weeds	Trace	16 ± 6	55 ± 7
Soliva sessilis	Trace	8 ± 1	Trace
Other weeds	Trace	26 ± 4	23 ± 5
Moss	Trace	9 ± 5	30 ± 6
Worm casts	0	7 ± 1	13 ± 2
Bare ground	11 ± 2	5 ± 3	7 ± 3
Total grasses	598 ± 20	438 ± 22	293 ± 27
Other species	16 ± 14	141 ± 16	227 ± 19

* Trace = less than 5 hits.

Herbage Yield (Table 3)

Dry-weight yields of "mat" plots ranged from 1,900 (Plot 2) to 3,300 lb per acre per annum (Plot 3). There were seasonal fluctuations in growth (high in November and December, low in June) but they were relatively small. As a result of the simple ammonium-sulphate treatment, Plot 2 was consistently the slowest growing plot. Seasonal differences in yield were less on the unfertilised plots.

As a group, fertilised "no mat" plots were of moderately high productivity. Compost (Plot 19) produced 9,400 lb with a pronounced peak of growth in the spring and a smaller peak in the autumn. Compost + NPK (Plot 21) and sodium nitrate + PK (Plot 31) produced 7,000 lb and dried blood + PK (Plot 29) produced 5,800 lb in a similar pattern. Superphosphate (Plot 1) also produced 5,800 lb but the spring peak was smaller and the winter and summer growth was more sustained. The NPKCa (Plot 5) plot grew throughout the year with relatively small peaks of growth to produce 5,200 lb.

TABLE 3. YIELDS OF DRY HERBAGE (lb/acre)

Growth Period	Fertilised		Not Fertilised
	Mat	No Mat	
17.7.51–11.9.51	375 ± 45	630 ± 50	170 ± 60
25.9.51–6.11.51	425 ± 75	1195 ± 85	335 ± 100
20.11.51–18.12.51	670 ± 140	2050 ± 150	600 ± 190
3.1.52–11.3.52	795 ± 95	1860 ± 100	545 ± 125
25.3.52–20.5.52	350 ± 45	715 ± 50	335 ± 60
3.6.52–1.7.52	91 ± 15	223 ± 17	116 ± 20
Year—17.7.51–1.7.52	2700 ± 360	6660 ± 390	2100 ± 480
15.7.52–26.8.52	96 ± 20	210 ± 20	88 ± 26
9.9.52–7.10.52	460 ± 50	1020 ± 50	390 ± 65
21.10.52–18.11.52	490 ± 95	1080 ± 100	590 ± 120
2.12.52–3.1.53	540 ± 110	1450 ± 120	470 ± 150
Total Period	4280 ± 540	10400 ± 580	3650 ± 720

Botanical Composition (Table 4)

In "mat" plots browntop and Chewing's fescue were dominant, while *Poa* spp. were the main weed grasses. In "no mat" plots browntop was dominant but other grasses, i.e. Yorkshire fog (*Holcus lanatus*, L.), *Poa* spp., and sweet vernal (*Anthoxanthum odoratum*, L.), and white clover (*Trifolium repens*, L.) were relatively important. In unfertilised plots, weeds were important.

In the summer, there was a moderate increase of Chewing's fescue in all plots, also of clovers and grasses other than browntop; whereas in the winter browntop increased, particularly in unfertilised plots.

TABLE 4. BOTANICAL COMPOSITION OF HERBAGE
(% dry weight)

	Fertilised		Not Fertilised
	Mat	No Mat	
Browntop	25.6 ± 4.0	49.1 ± 4.3	38.8 ± 5.3
Chewing's fescue	68.7 ± 4.3	10.8 ± 4.6	23.0 ± 5.7
Other grasses	Trace	14.2 ± 0.9	7.5 ± 1.1
White clover	2.7 ± 1.4	17.0 ± 1.5	9.4 ± 1.8
Other clovers	Trace*	2.7 ± 0.4	4.0 ± 0.5
Weeds	Trace	6.0 ± 1.5	17.3 ± 1.8

* Trace = less than 2%.

Chemical Composition (Table 5)

The calcium content of "mat" herbage was consistently less than that of other herbage. Herbage from Plot 2 (ammonium sulphate 6 cwt) contained about 0.31% phosphorus, which was similar to unfertilised. The nitrogen content of fertilised "no mat" herbage was consistently higher than that of other herbage. There were no clear changes due to season.

TABLE 5. CHEMICAL COMPOSITION OF HERBAGE—SUMMER 1951
(% dry weight)

	Fertilised		Not Fertilised
	Mat	No Mat	
Total ash	10.70 ± 0.20	11.30 ± 0.20	13.80 ± 0.30
Insol. ash	4.20 ± 0.30	3.50 ± 0.10	6.90 ± 0.40
Ca	0.41 ± 0.35	0.93 ± 0.38	0.75 ± 0.46
P	0.49 ± 0.26	0.53 ± 0.28	0.31 ± 0.35
K	2.31 ± 0.73	2.54 ± 0.79	2.26 ± 0.97
N	3.37 ± 0.59	4.19 ± 0.64	3.39 ± 0.78

Worm Counts, Soil pH, and Soil Resistance to Penetration (Table 6)

In each of the annual worm counts, there was only an occasional worm in "mat" plots, whereas there were many in "no mat" plots, the highest numbers being in Plots 1 and 29. Despite the compost treatment there were few worms in Plot 21. Activity at the soil surface extended from April to November.

The "mat" plots were consistently more acid than "no mat" plots. Acidity increased from the winter to the summer, from 4.7 ± 0.12 to 4.4 ± 0.11 in "mat" plots, from 6.2 ± 0.13 to 5.5 ± 0.12 in fertilised "no mat" plots, and from 6.3 ± 0.16 to 5.5 ± 0.15 in unfertilised plots.

TABLE 6. SOIL pH, EARTHWORM COUNTS, AND RESISTANCE TO PENETRATION

	Fertilised		Not Fertilised
	Mat	No Mat	
pH	4.6 ± 0.9	5.9 ± 1.0	6.0 ± 1.2
Worm No./cubic foot	Trace	44 ± 7	43 ± 12
Soil resistance to penetration lb/sq. in.	404 ± 11	170 ± 12	154 ± 14

"Mat" plots were very resistant to penetration at all times, whereas "no mat" plots offered little resistance when wet. The mat offered considerable resistance to penetration, but, when it was stripped back and the exposed soil tested, it was clear that most of the resistance was due to the soil itself.

Soil Carbon and pH (Table 7)

If the mat is excluded, it can be seen that the carbon content of "mat" soil was consistently a little less than that of "no mat" soil. Although not shown, the difference was large in a shallow soil layer within 1 in. of the surface. The pH of the "mat" soil was consistently lower than that of "no mat" soil, the difference being large near the surface and small below the 9 in. depth.

TABLE 7. SOIL pH AND CARBON CONTENT (%) OF PROFILES IN PLOTS 2A AND 1A

Layer	Fertilised			
	Mat (2A)		No Mat (1A)	
	pH	C	pH	C
Top mat	4.67 ± 0.06	24.8 ± 1.3		
Bottom mat		6.6 ± 0.9		
0– 1 in.	5.13 ± 0.07	3.00 ± 0.10	6.05 ± 0.11	4.03 ± 0.20
1– 4 in.	5.28 ± 0.08	2.29 ± 0.09	6.19 ± 0.03	2.49 ± 0.03
4–11 in.	5.87 ± 0.04	1.06 ± 0.09	6.16 ± 0.04	1.39 ± 0.09

Soil Volume Weight (Table 8)

Near the soil surface, volume weight was higher in the "mat" plots than in the "no mat" plots. This difference did not occur at the lower levels.

TABLE 8. SOIL VOLUME WEIGHTS IN "MAT" AND "NO MAT" PROFILES (g/cm³)

Layer	Mat	No Mat (+ and — Fertiliser)
0 – 1¼ in. or 0–2 in.	* 1.19 ± 0.04	0.96 ± 0.02
1⅛– 2⅜ ,,	1.23 ± ,,	
1 – 3 ,,	1.19 ± ,,	1.07 ± ,,
2 – 4 ,,	1.17 ± ,,	1.15 ± ,,
5 – 7 ,,	1.23 ± ,,	1.24 ± ,,
8 –10 ,,	1.29 ± ,,	1.32 ± ,,

* Sampled immediately beneath the mat.

Soil Moisture (Table 9)

After heavy rain, when water was held loosely in the mat, the moisture content of "mat" soil was consistently less than in "no mat" soil, notably near the surface.

TABLE 9. SOIL-MOISTURE CONTENT OF PLOT PROFILES—WINTER 1952 (g/100 g dry soil)

Layer	Fertilised		Not Fertilised
	Mat	No Mat	
Top mat	173.0 ± 12.0		
Bottom mat	71.7 ± 12.0		
0 – 0.5 in.	36.0 ± 1.4	63.0 ± 1.8	57.0 ± 2.7
0.5– 1 ,,	30.3 ± ,,	56.5 ± ,,	50.5 ± ,,
1 – 2 ,,	30.3 ± ,,	48.0 ± ,,	47.0 ± ,,
2 – 3 ,,	29.5 ± ,,	42.0 ± ,,	38.0 ± ,,
3 – 4 ,,	30.7 ± ,,	39.5 ± ,,	31.5 ± ,,
4 – 6 ,,	30.3 ± ,,	39.0 ± ,,	33.0 ± ,,
6 – 8 ,,	25.5 ± ,,	30.5 ± ,,	30.5 ± ,,
8 –10 ,,	21.0 ± ,,	25.0 ± ,,	26.5 ± ,,

Observations

The plant cover of "mat" plots was more finely leaved and uniform than that of the others and appeared to be more tolerant of treading damage. Most "no mat" plots, particularly plots 1, 29, and 31, were observed to be damaged by treading when the soil was wet.

The protective turf mat was composed of intertwined stolons, roots, dead leaves, sheath tissue, and partly decomposed material. Chewings fescue appeared to have produced much of the sheath tissue.

The mat was usually moist, but if it dried it was difficult to wet; that on Plot 8 was most susceptible to drying.

Worms present were mainly *Allolobophora caliginosa* and *Lumbricus rubellus,* and sometimes *Octoclasium* spp.

The structure of "mat" soils appeared to be poorly developed, with small aggregates, whereas in "no mat" soils the aggregates were larger.

Oxycanus (Porina) damage occurred principally in the "mat" plots.

DISCUSSION

The experimental layout ignored soil variation. However, by grouping the plots in three groups according to turf type—"Mat", Fertilised "No Mat", and Unfertilised "No Mat"—it was possible to calculate means and standard errors of reasonable validity. There were large differences between these groups in spite of the variable nature of the data within each group.

The most striking treatment effect was the development of the turf mat, which was 1–1¼ in. thick in 1951. It appeared wherever the surface soil pH was 5.0 or less, and where there were few organisms to incorporate the plant tissue into the soil. There were few earthworms in Plot 21, and the compost accumulated on the surface, whereas in the other "no mat" plots there was intense earthworm activity during the winter and spring, and there was no mat development. Soil structure under the "mat" appeared to be poor, and the mat was wet for long periods. The lower temperatures and anaerobic conditions that this implies, together with the salt effect in the mat resulting from the regular applications of ammonium sulphate, probably slowed down the rate of decomposition. The results of proximate analyses (not shown) suggested that, because of its relatively undecomposed state, the top mat was similar to an immature peat, which agrees with Fraser's (1950) conclusion. It is notable that browntop and Chewing's fescue were co-dominant in the "mat" swards.

Growth on all plots responded to season, to a varying degree, the spring and autumn being the most productive periods. The plants had become adapted to regular mowing by forming many small tillers rather than fewer large ones. They were never subjected to intensive wear. Fertilised "no mat" plots were very productive, Plot 31 (vigorous clover growth) and Plots 19 and 21 (weedy compost implying reseeding mulching, etc.) yielding 7,000–9,400 lb of dry herbage. The lower productivity of "mat" plots may have been caused by nutrient deficiency induced by inadequate fertilising or by the effects of soil pH on availability, and microbial activity. Yet the turf, notably that on Plot 3, appeared to be persistent.

Although the "mat" and "no mat" herbage differed in species composition, it was reasonable to expect the reduced calcium content of the former. By normal standards the cation-exchange capacity of this alluvial soil was about average (17–22 m.e.%, Metson and Gibbs 1946), but with acidification to about 9 in. depth, a significant reduction in cation saturation and availability could have occurred. It is possible that applications of potassic fertilisers heightened this effect. The low nitrogen content of "mat" herbage might be due in part to reduced mineralisation of soil nitrogen but it is difficult to reconcile this with the regular applications of fertiliser nitrogen. Thus it appears that nutrient deficiency may have restricted the growth of "mat" plots.

Browntop and Chewing's fescue, fertilised with superphosphate and ammonium sulphate (Plot 3), produced a turf apparently suited to sporting requirements, in agreement with Hartwell and Damon (1917) and Dawson and Greig (1933). But the turf was susceptible to summer drought, which, together with the induced low fertility, possibly caused the dominance of Chewing's fescue. The persistence of browntop could have been due to protracted wet conditions in the mat during the autumn-spring period. Hall and Moses (1931) recommended the use of ferrous sulphate to assist mat formation, but this treatment (Plot 8) increased the susceptibility of the turf to drought, and browntop was greatly reduced.

The high degree of dispersion of the browntop among the Chewing's fescue in "mat" swards, produced a uniform turf and contrasted with pronounced species aggregation in "no mat" plots, particularly in unfertilised plots. Most of the species in fertilised "no mat" plots were volunteers, and, under the ruling conditions of severe, repeated defoliation, were restricted in their ability to disperse through the turf. Sweet vernal, Yorkshire fog and *Poa* spp. persisted, but, in the face of competition from browntop and clovers, failed to disperse. When resistance to wear, freedom from worm casts, and soil stability are also considered, it is clear that "mat" turfs are more suited to most sporting requirements.

By judiciously applying fertilisers, a satisfactory turf, with a thin mat, might be maintained on a moderately dense soil which is capable of absorbing adequate water for sustained grass growth. In practice this is being done with fair success in most districts in New Zealand by periodically including a lime dressing in the fertilising programme. However, the need for a stable soil mass with good structure persists, and perhaps soil additives may facilitate this, particularly in the absence of worms.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of the staff of Grasslands Division and Mr. A. C. Glenday, Applied Mathematics Laboratory, Department of Scientific and Industrial Research.

REFERENCES

DAWSON, R. G.; GREIG, R. 1933: *J. Bd. Greenkeep. Res. 5:* 169-73.
FRASER, G. K. 1950: *Ibid. 7:* 322-34.
HALL, T. D.; MOSES, D. 1931: *S. Afr. J. Sci. 28:* 180-201.
HARTWELL, B. L.; DAMON, S. C. 1917: *Rhode Is. agric. Exp. Sta. Bull. 170.*
LEVY, E. B.; MADDEN, E. A. 1933: *N.Z. J. Agric. 46:* 267-79.
——————1940: The Greens Res. Comm., *N.Z. Golf Ass. 5th annu. Rep.* pp. 9-15.
METSON, A. J.; GIBBS, H. S. 1946: *N.Z. J. Sci. Tech. A27:* 413-26.
SEARS, P. D.; NEWBOLD, R. P. 1942: *Ibid. A24:* 36-61.

THE EFFECT OF DIFFERENT PHOSPHATE FERTILISERS ON SOIL pH AND THE CONSEQUENT EFFECT ON PHOSPHATE RETENTION

By W. M. H. Saunders, Soil Bureau, Department of Scientific and Industrial Research, Wellington, N.Z.

(*Received for publication, 5 June 1958*)

Summary

A comparison has been made of the degree of phosphate retention by yellow-brown loam of different phosphate fertilisers—superphosphate, double superphosphate, potassium dihydrogen phosphate and diammonium phosphate. The soils used were of low, medium and high phosphorus status. The addition of the phosphate to the soil suspension alters the pH, and the differences between the pH values of the suspensions are sufficient to account for the differences in phosphate retention.

To determine how long these differences in pH persist, a low and a high phosphorus status soil were topdressed in a laboratory experiment at 90 lb P/acre and the pH of the surface soil measured over a period of three months. Changes of over one pH unit were recorded after topdressing, and it is considered that, even under light topdressing in the field, these pH changes would occur in the immediate vicinity of the phosphate particles and affect the concentration of phosphate in the soil solution and the utilisation of the applied phosphate.

Introduction

It is well established that the retention of phosphate by soils changes with pH but it appears that in comparisons of retention of phosphate from different phosphate fertilisers, no report has been made wherein the differences are related to the effect of the fertilisers on soil pH. A comparison has been made, therefore, of the retention of phosphate from four fertilisers—superphosphate, double superphosphate, potassium dihydrogen phosphate, and diammonium phosphate—and at the same time the pH of the soil:phosphate systems was measured. The soil used is a member of the yellow-brown loam group (New Plymouth black loam) formed from andesitic volcanic ash and has a very high capacity to retain phosphate. (New Zealand Soil Bureau 1954.)

Methods and Results

Comparison of Phosphate Retention

Six soil samples were collected from 0–2 in. under old pasture from fields which had low, medium, and high phosphorus status. Two samples were collected at each phosphorus level. Some chemical analyses of the soils are set out in Table 1.

2 g of the air-dried soil ($<$ 2 mm) were shaken wtih 20 ml of water or phosphate solution (50 p.p.m. P) for 24 hr at 20°c, filtered and the soil washed with small volumes of water until the total filtrate amounted to 100 ml. The pH of each soil suspension was measured

TABLE 1.—CHEMICAL ANALYSES OF THE NEW PLYMOTH BLACK LOAM OF LOW, MEDIUM, AND HIGH PHOSPHORUS STATUS
(Each value reported is the mean from two soils)

P Status	pH	CEC m.e.%	%BS	Exch. Ca m.e.%	N %	C/N	Total P mgP%	*Truog P mgP%
Low	5.8	39.9	51	15.0	1.06	13	230	1.2
Medium	5.8	43.0	61	21.6	1.15	12	323	2.5
High	6.1	46.6	77	30.2	1.06	14	370	6.1

* P soluble in Truog reagent.

with a glass electrode and the inorganic phosphate content of each filtrate determined colorimetrically (Williams and Stewart 1941).

The inorganic phosphorus content of the filtrates, the percentage of the added phosphate remaining in solution, and the pH of the soil:phosphate suspensions are set out in Table 2. Each value is the mean from two samples at each phosphorus level. All differences are significant at the 5% level. The concentration of phosphate in the filtrates is very low even when phosphate has been added. For each phosphate carrier, the phosphate in solution increases, and phosphate retention decreases, as the phosphorus status of the soil improves. At

TABLE 2. INORGANIC PHOSPHORUS IN FILTRATE, % OF ADDED PHOSPHATE REMAINING IN SOLUTION, AND pH OF THE SOIL: PHOSPHATE SUSPENSIONS

Soil Phosphorus Status	Water	Super	Double Super	KH_2PO_4	$(NH_4)_2HPO_4$
	Inorganic P Content of Filtrate (p.p.m. P)				
Low-P	0.002	0.015	0.125	0.285	0.440
Medium-P	0.010	0.055	0.150	0.375	0.490
High-P	0.025	0.230	0.420	0.580	0.760
	% of Added Phosphate Remaining in Solution				
Low-P	—	0	1	3	4
Medium-P	—	1	2	4	5
High-P	—	2	4	6	8
	pH of Soil: Phosphate Suspension				
Low-P	5.8	5.5	5.6	5.9	6.2
Medium-P	5.9	5.6	5.8	6.0	6.2
High-P	6.2	5.9	6.1	6.2	6.4

each phosphorus status, the amount of phosphate in solution increases and retention of the added phosphate decreases, in the order: superphosphate, double superphosphate, potassium dihydrogen phosphate, diammonium phosphate. This is in agreement with results of penetration and diffusion experiments in which ammonium phosphates have been found to penetrate and diffuse further than superphosphates (Heslep and Black 1954; Midgley 1931; Spencer and Stewart 1934).

The pH of the soil:phosphate suspensions increases in the same order as the degree of phosphate retention decreases, i.e., superphosphate, double super, KH_2PO_4 and $(NH_4)_2HPO_4$. The differences of pH and of concentration of phosphate in solution with the various phosphates are illustrated for the low–P and heavy–P soils in Fig. 1 by curves 1 and 2 respectively. Curve 3 is a mean of six pH-phosphate retention curves for these three pairs of soils determined according to Mattson *et al.* (1950), and may be compared with curves 1 and 2.

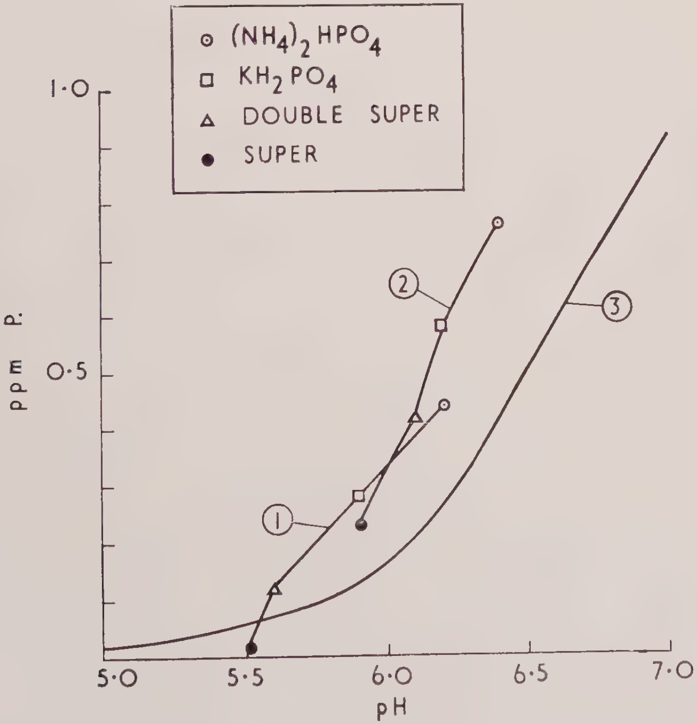


Fig. 1.—The concentration of phosphate and pH of soil suspensions with different phosphate fertilisers added. Curve 1, heavy-P soils; Curve 2, low-P soils; Curve 3, mean for six pH-phosphate retention curves for the New Plymouth black loam.

This curve shows the decrease of phosphate retention with increasing pH above pH 5. Because of different experimental conditions, namely, a smaller soil:solution ratio (1:50) and, smaller amount of added phosphate (44 mg P/100 g), curve 3 lies slightly below curves 1 and 2. It is clear that the differences in pH between the different phosphate suspensions are sufficient to account for the differences in the amount of phosphate in solution and hence in the degrees of phosphate retention.

In addition, however, the soluble calcium introduced into the systems in the superphosphates is causing some increased retention of the phosphate as shown by the concentration of phosphate in the superphosphate systems being lower than would be expected from the shape of curve 3. The concentration of calcium in the 1:10 soil:superphosphate solution was approximately 0.013 and 0.0065 N for the ordinary and double superphosphate respectively. This concentration is sufficient to suppress the Donnan distribution of the phosphate about the soil colloid (Miller 1954), this suppression appearing as increased retention of the added phosphate. There is no need to suggest specific calcium to phosphate bonding occurring in the moderately acid systems, pH 5.5 to 6.1.

Although the exchangeable Ca content of the high–P soils is twice that of the low–P soils, phosphate retention is lower in the high–P soils and this is most likely due to the higher pH and the higher phosphorus content of the latter soils. It is evident that in these moderately acid soils exchangeable calcium has little effect on phosphate retention. pH is the dominant factor determining the degree of phosphate retention in this soil and it is by their effect on the soil pH that the various phosphate carriers determine the relative degree to which their phosphate is retained.

Effect of Topdressing on pH of Soil

The increase in pH cause by diammonium phosphate is contrary to field experience which is that prolonged use of ammonium phosphates acidifies soils (Rogers *et al.* 1953; Ensminger and Pearson 1957). This inconsistency was investigated by putting 35 g of one of the low–P soils and one of the high–P soils in small pots (7 cm diam. and 1 cm depth of soil) and topdressing them at a rate of 1 mg P/cm^2 (90 lb P/acre) with diammonium phosphate and the two superphosphates. Before topdressing, the soils were held at field capacity for 7 days and then after topdressing they were watered by adding 50 ml of water each week, 10 ml being added each day from Monday to Friday. The pots were kept in the laboratory where the temperature ranged from 15° to 20°C. The pH of the top 3 to 4 mm of the soil was measured using spearpoint glass and calomel electrodes. For each pH determination, six readings were made with the electrodes in different positions. The mean value of the six measurements was recorded. The changes of the pH of the surface soil were followed for 130 days and are shown in Fig. 2.

The pH values of the soils at zero time were 6.1 and 6.8 respectively for the low–P and the high–P soils. These values were much higher than those obtained in a 1:2.5 soil:water suspension (5.8 and 6.1). The rise in pH continued during the first week after topdressing and its most probable cause is the occurrence of ammonification. Over and

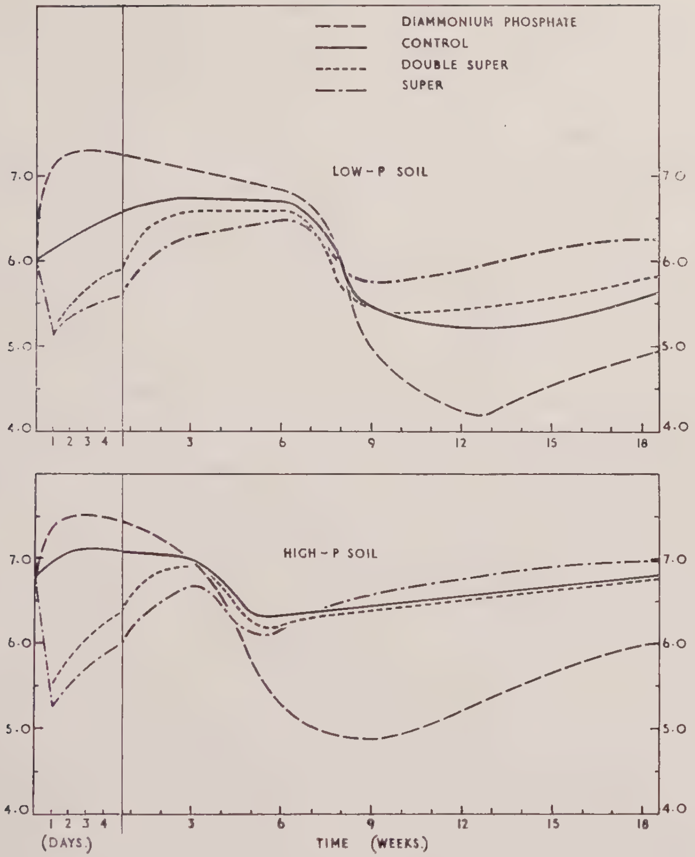


Fig. 2.—Changes of pH of soils following topdressing with different phosphate fertilisers.

above this rise of pH, topdressing with ammonium phosphate increased the pH by about 0.5 units in both soils. Topdressing with the superphosphates acted against the general rise in pH and lowered the pH by about 1 unit. These changes occurred within 24 hours in both soils. In the following weeks the changes in pH were similar in direction for both soils but differed in the period of time after topdressing at which they occurred. During a period of 6 weeks for the low-P and 3 weeks for the high-P soil the pH of all phosphate treatments moved toward that of the control soils and then the pH of all treatments, including the control, fell rapidly by about a pH unit over a period of 2 weeks. After this fall the pH of all treatments, except the diammonium phosphate, increased slowly over the remaining period of the experiment. Superphosphate appreciably raised the pH of the low-P soil above that of the control but had no effect on the pH of the high P soil. In accord with the greater Ca:P ratio of the normal superphosphate, it has raised the soil pH above that of the soils topdressed with double superphosphate. The pH of the soils treated with diammonium phosphate, however, continued to fall for another 2 to 3 weeks and reached the very low values of 4.2 and 4.9 in the low–P and high–P soils respectively. The pH of these soils then rose steadily toward that of the other treatments.

The initial decrease of pH in the soils treated with the superphosphates is due to the acid nature of the fertilisers and to the replacement of exchangeable H^+ ions by Ca^{2+} ions from the fertilisers, especially from ordinary superphosphate. This difference between the two superphosphates is illustrated by the more rapid recovery of the pH in the soils treated with double superphosphate. The initial increase of pH after topdressing with diammonium phosphate is due to its neutral to alkaline reaction.

It is probable that ammonification occurred during the first week of the experiment and it appears that, after a lag period, nitrification occurred in all treatments with a consequent decrease in pH. This occurred to the greatest extent in the soils treated with diammonium phosphate because of the greater amount of ammonium ions present. The shorter lag period for the high fertility high–P soil is consistent with this fall in pH being due to bacterial nitrification. After nitrification ceased, the acidity was leached away and the pH increased again, but it is probable that the pH of the soils treated with diammonium phosphate remains below that of the other treatments. This is indicated by field evidence.

CONCLUSIONS

The preceding results show, first, that the differences in phosphate retention between various phosphate fertilisers are brought about by the effect of the fertilisers on the soil pH; and, second, that over a period of time the effect of the fertiliser on pH may change. It is considered that, in view of the dominant effect of pH on the concentration of phosphate in solution in the first experiment, it may be reasonably assumed that the pH changes occurring in the pot experiment were accompanied by corresponding changes in the concentration of phosphate in the soil solution. It was not possible to measure directly, without disturbing the soil in the pots, the effect of the pH changes on the concentration of phosphate in the soil solution. The concentration of phosphate in the leachates from the pots could not be accepted as a satisfactory measure of the effect of the pH changes because the leachates passed through 6 mm of soil under the soil whose pH was measured, and the effect of this lower layer of soil on the composition of the leachate was not known. The changes in soil pH and level of soluble phosphate brought about by the different fertilisers in the present soil may also be expected to occur in other acid soils where phosphate retention decreases as their pH is raised. The extent of the changes would depend on the buffer capacity and the nature of the phosphate-retaining complex of the soils.

When the comparison of phosphate retention is made with only a short period of contact, as in the first experiment above, diammonium phosphate maintains a higher concentration of phosphate in solution than do either of the superphosphates. It therefore shows greater diffusion and penetration in the soil. If the comparison is carried out after nitrification has occurred the results from the second experiment indicate that the superphosphates would show a higher phosphate concentration in the soil solution because of a higher pH.

As the ratio of phosphate to soil used in the second experiment is probably no greater than would occur in the immediate vicinity of a phosphate fertiliser particle in the soil, changes of pH of the same magnitude as shown in Fig. 1 may be expected to occur near the particle and influence the utilisation of the fertiliser phosphorus. For example, the author has found in a field trial on the present soil, comparing the effect of increasing rates of double superphosphate on pasture growth, that immediately after topdressing growth is depressed, the effect increasing with rate of topdressing. However, after three or four weeks the pasture is responding to the added phosphate. Ensminger and Cope (1947) have reported that, after prolonged use of ammonium phosphate on a moderately acid soil without liming, production of cotton seed fell below that from no-phosphate control plots, and in a comparison of the residual effects Ensminger and Pearson (1957) found considerably less residual effect from ammonium phosphate than from superphosphate. They ascribed the difference to the lower pH of the soil treated with ammonium phosphate. The residual acidity from ammonium phosphates can, however, be avoided by adequate liming. One further point about the field comparisons of ammonium phosphates and superphosphates is worth mentioning. In order to equalise the nitrogen levels in such comparisons it is the common practice to add an ammonium salt, such as ammonium sulphate, with the superphosphate. This salt may be expected to undergo nitrification in the same manner as ammonium phosphate and thereby reduce the utilisation of phosphate from the superphosphates.

REFERENCES

ENSMINGER, L. E.; COPE, J. T. 1947. Effect of Soil Reaction on the Efficiency of Various Phosphates for Cotton and on the Loss of Phosphorus by Erosion. *J. Amer. Soc. Agron. 39:* 1-11.

ENSMINGER, L. E.; PEARSON, R. W. 1957. Residual Effect of Various Phosphates as Measured by Yields, P^{32} Uptake and Extractable Phosphorus. *Proc. Soil. Sci. Soc. Amer. 21:* 80-4.

HESLEP, J. M.; BLACK, C. A. 1954. Diffusion of Fertiliser Phosphorus in Soils. *Soil Sci. 78:* 389-401.

MATTSON, S.; ALVSAKER, E.; KOUTLER-ANDERSSON, E.; BARKOFF, E.; VAHTRAS, K. 1950. Phosphate Relationships of Soil and Plant, VI. The Salt Effect on Phosphate Solubility in Pedalfer Soils. *LantbrHögsk. Ann. 17:* 141-60.

MIDGLEY, A. R. 1931: The Movement and Fixation of Phosphates in Relation to Permanent Pasture Fertilisation. *J. Amer. Soc. Agron. 23:* 788-99.

MILLER, R. B. 1954: Phosphate Relationships of Soil and Plant. XII. A Note on Salt and Dilution Effects in the Brännalt Podzol Soil. *LantbrHögsk. Ann. 21:* 177-87.

NEW ZEALAND SOIL BUREAU 1954: General Survey of the Soils of North Island, New Zealand. *N.Z. Soil Bur. Bull. n.s. 5:* p. 140.

ROGERS, H. T.; PEARSON, R. W.; ENSMINGER, L. E. 1953: Comparative Efficiency of Various Phosphate Fertilisers. "Soil and Fertiliser Phosphorus in Crop Nutrition; Agronomy IV," pp. 189-236. *Academic Press, New York.*

SPENCER, V. E.; STEWART, R. S. 1934: Phosphate Studies. I. Soil Penetration of Some Organic and Inorganic Phosphates. *Soil Sci. 38:* 65-80.

WILLIAMS, E. G.; STEWART, A. B. 1941: The Colorimetric Determination of Readily Soluble Phosphate in Soils. *J. Soc. Chem. Ind., Lond. 60:* 291-7.

STUDIES OF GRAZING OF CROP FORAGES BY SHEEP

II. WASTAGE OF RAPE BY GRAZING WETHERS

By A. F. Greenall*, Crop Research Division, Department of Scientific and Industrial Research, Lincoln.

(*Received for publication, 24 April 1958*)

Summary

Wastage associated with the grazing of rape by harnessed wethers was measured by forage sampling and by total faecal collection, to estimate intake from the consumption/excretion ratio. Blocks were grazed in accordance with lamb fattening practice.

Stubble residues wasted amounted to 18% of both the dry matter and organic matter offered. The wastage of starch equivalents was slightly lower.

Grazing wastage averaged 17% of the dry matter and 20% of the organic matter. The wastage of starch equivalents was slightly higher.

The mean digestion wastage was 10% of the dry matter and 7% of the organic matter of the rape offered.

Only 56% of the rape dry matter offered was used for growth and maintenance.

There was differential wastage of proximate constituents amounting to 95% of ether extract, 67% of crude protein and 38% of crude fibre. The wastage of nitrogen-free extract, by far the largest fraction, was similar to that of organic matter.

The results were in agreement with the hypothesis that, over the normal range of yield, a doubling of yield is accompanied by a quadrupling of grazing wastage. There appeared to be relationships between yield and other sources of wastage and between stubble wastage and grazing intensity.

Further investigation of factors associated with crop and animal management is warranted if fuller utilisation of crop forages is to be achieved. It is evident from the results of this experiment that yields obtained from cutting cannot be safely used to predict animal production.

INTRODUCTION

In New Zealand, sheep are fed on herbage or forage crops for most or all of the year. In countries with more rigorous climates hand feeding is necessary, and its use facilitates direct measurement not only of crop yield, but also of quality and utilisation. Under these conditions utilisation may be nearly complete. When animals graze, direct measurement of intake is impossible, but several methods of indirect measurement have been employed.

The technique used most frequently has been the determination of live-weight gain, from which, by the reverse use of Wood and Woodman feeding tables (1930), the intake of "feed units" is calculated.

---

* Now Chief Soil Conservator, Otago Catchment Board, Dunedin.

N.Z. J. agric. Res. 1: 683–693.

This is checked against crop yield expressed as "feed units"—the product of dry-matter yield and standard starch-equivalent values (Brown 1954). This method is at best an approximation, for three reasons:

(1) The maintenance requirement of sheep in metabolism cages probably differs from that of free-grazing sheep.

(2) Grazing wastage is difficult to measure and so is rarely taken into consideration.

(3) Weight gains cannot be measured accurately over short grazing periods (Anon. 1952; Brown 1954). The techniques of Lancaster (1954), Reid *et al.* (1950) and Raymond *et al.* (1956) and of Van der Kley (1956) have been mainly employed for pasture research. Little work has been done on the grazing of annual forage crops by sheep.

It can be observed in Fig. 1 that wastage occurs during biting and chewing and through trampling and fouling and the leaving of stubble (Peterson *et al.* 1956). Accordingly less forage is consumed than is offered, and that consumed is different from that offered (Greenall 1958). The present study was undertaken to determine the amount and composition of rape wasted in grazing by sheep.

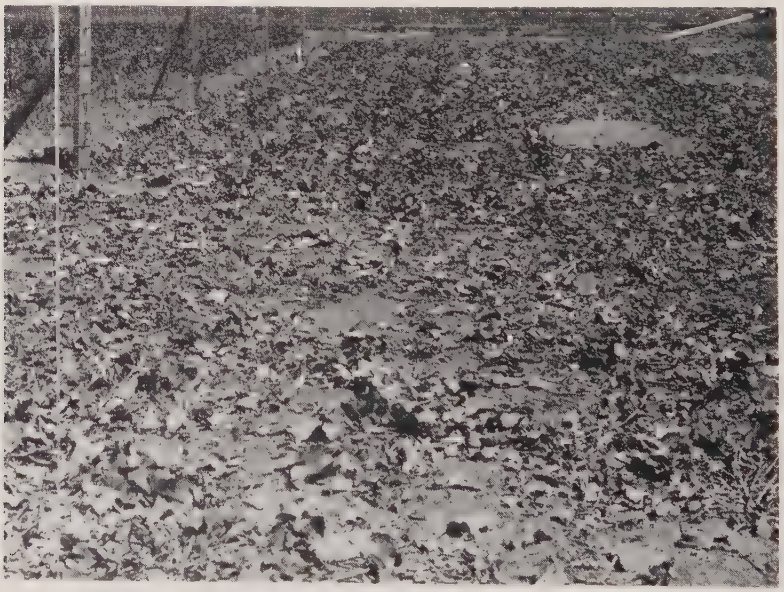


Fig. 1. Block III on the 6th day of grazing with the ungrazed part in the left background.

—*Photo: J. Webb*

METHODS

Rape was grown for grazing in four blocks as previously described (Greenall 1958). The amount of rape offered for consumption was determined by sampling the whole area of each block just before grazing commenced, and sampling the ungrazed portion of each block just after grazing finished. The forage was cut at ground level. The yield was computed from the mean of these two samples, thus allowing for yield increments from growth during the period of grazing, or yield losses by wilting or by insect pests. Each block was divided into two parts of similar yields of dry matter (D.M.) per unit area. The larger part was sufficient to supply grazing *ad lib.* for 16 two-tooth halfbred wethers for about eight days. The remaining part was the source of the feed for two wethers eating ungrazed rape in digestibility experiments. The sub-blocks were each grazed once with the same 16 wethers, harnessed for faecal collection. Meyer *et al.* (1956) reported that harnessing one third of a group of animals for faecal collection gave a reliable measure of intake, but harnessing the whole group should give greater precision. In addition, rape was selected from the grazing part of each block for feeding to the two wethers eating selected rape in the digestibility experiments previously described (Greenall 1958). The amount of rape offered to the grazing wethers was calculated, adjusting for the difference between grazing period and sampling interval and for the feed removed for the digestibility experiments.

Composite samples of the rape offered for grazing in blocks II and III were analysed for organic matter (O.M.) and proximate constituents. The units of starch equivalents (S.E.) offered were then calculated by the method outlined in the preceding paper of this series (Greenall 1958). The mean O.M. percentage of these two blocks was used to estimate, from the measured weight of D.M., the O.M. offered for grazing in blocks I and IV.

The weight of D.M. remaining as stubble after grazing was determined by sampling. O.M., proximate constituents, and S.E. were calculated after analysis, in a manner similar to that employed for food offered.

The weight of rape apparently consumed, in terms of D.M., O.M., proximate constituents, and S.E., was then determined from the difference between the rape offered for grazing and the stubble remaining after grazing.

The weight of rape actually consumed, as distinct from the rape apparently consumed was found from a consumption/excretion ratio technique first used by Garrigus (1935). Garrigus and Rusk (1936) claim that this technique of measuring intake involves errors totalling less than 5%. The consumption/excretion ratio of rape selected from the grazing part of each block was determined in digestibility experiments. The faeces excreted by the grazing wethers were collected, and the weight of rape consumed was computed. The proximate constituents of a bulk sample of the faeces were determined. These data, together with the apparent digestibility coefficients of D.M., O.M., and proximate constituents, enabled the weight of D.M., O.M., proximate constituents, and S.E. eaten to be calculated. This technique was used by Woodman *et al.* (1937), by Kane *et al.* (1953) and by Brisson *et al.* (1954) for pasture. These authors do not question the accuracy claimed by Garrigus and Rusk but mention the laborious nature of the technique and question the validity of the assumption that the feed selected by hand is truly representative of that eaten by grazing animals.

RESULTS

Estimates of the weight of rape offered for eating, of stubble residues, of rape apparently consumed, of grazing wastage of rape actually consumed, and of digestibility wastage, were obtained for each block and expressed as D.M. and O.M. In addition, for blocks II and III, weights were expressed as proximate constituents and S.E.

The percentage wastage of D.M., O.M., proximate constituents, and S.E., is shown in Table 1.

TABLE 1. WASTAGE OF RAPE BY GRAZING WETHERS

Block No.	Constituent	% Wastage			
		Stubble	Grazing	Digestion	Total
I	Dry matter	20.6	16.5	8.8	45.9
	Organic matter	20.6	17.4	6.7	44.7
II	Dry matter	23.6	23.9	7.8	55.3
	Organic matter	23.6	23.0	5.4	52.0
	Starch equivalents	23.2	23.7		46.9
	Crude protein	22.9	33.6	12.8	69.3
	Crude fibre	27.6	10.9	9.6	48.1
	Ether extract	13.8	61.1	23.2	98.1
	Nitrogen-free extract	23.2	22.4	2.9	48.5
III	Dry matter	12.9	19.9	9.2	42.0
	Organic matter	12.9	24.2	6.4	43.5
	Starch equivalents	12.6	26.1		38.7
	Crude protein	10.8	40.1	13.6	64.5
	Crude fibre	16.9	1.8	8.6	27.3
	Ether extract	7.9	47.4	36.8	92.1
	Nitrogen-free extract	12.7	24.8	3.8	41.3
IV	Dry matter	13.5	8.0	12.6	34.1
	Organic matter	13.5	13.8	7.8	35.1
Mean	†Dry matter	17.6	17.1	9.6	44.3
	†Organic matter	17.6	19.6	6.6	43.8
	*Starch equivalents	17.9	24.9		42.8
	*Crude protein	16.8	36.9	13.2	66.9
	*Crude fibre	22.3	6.3	9.1	37.7
	*Ether extract	10.8	54.3	30.0	95.1
	*Nitrogen-free extract	17.9	23.6	3.3	44.8

* Blocks II and III.
† Blocks I to IV.

Stubble Residues

The mean wastage of D.M. and of O.M. in stubble residues was 17.6%, with a range from 12.9 to 23.6%. In blocks II and III the wastage of S.E. approximated that of D.M. and O.M. For the purpose of computing the S.E. value of the stubble it was assumed that the proximate constituents of the stubble would be digestible to the same extent as the proximate constituents of selected rape. While this assumption is a reasonable one (Greenall 1958) the calculated value may be high, so that loss of S.E. in stubble may be overestimated. The

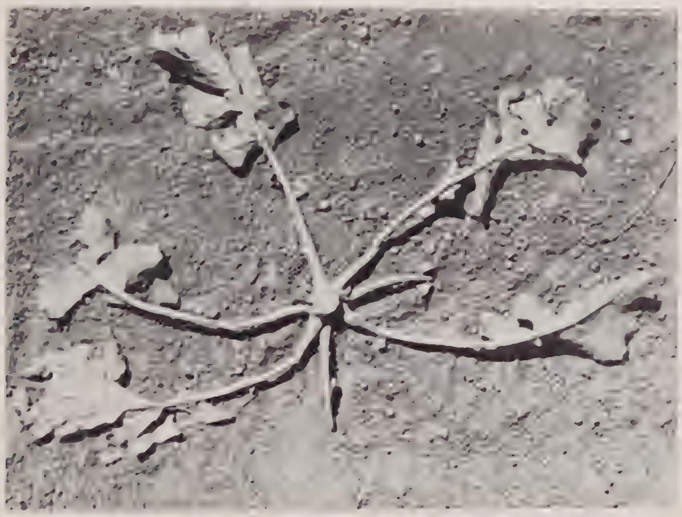


Fig. 2.—Hoof damage to the petioles of a rape plant, the laminae of which subsequently die.

—*Photo: J. Webb*

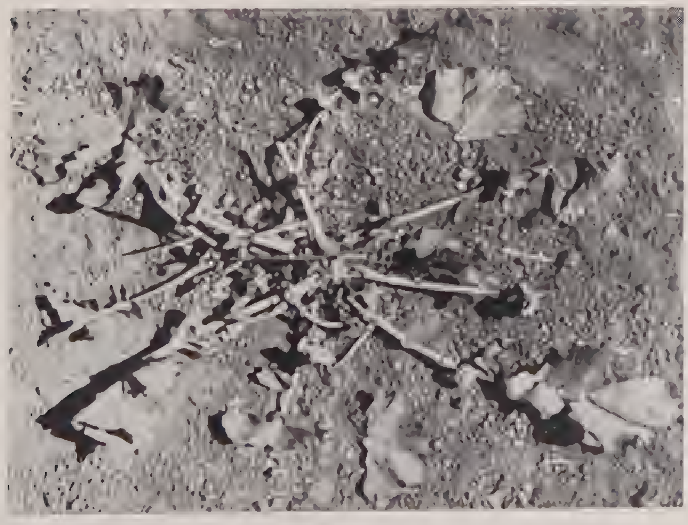


Fig. 3.—Portions of the laminae of rape leaves which have been dropped by sheep during grazing.

—*Photo: J. Webb*

wastage of nitrogen-free extract (N.F.Ex.) was similar to that of D.M. and O.M. whereas that of crude fibre (C.F.) was higher, and that of crude protein (C.P.) and ether extract (E.Ex.) was lower. This was due to the difference in composition between leaf and stem.

Grazing Wastage

It can be readily observed, and has been recently reported by Peterson *et al.* (1956) and Greenall (1958), that ruminants waste forage mainly by dirtying and destroying prostrate and dropped leaves. That this is true for rape is shown in Figs. 2 and 3. Where forages are grazed in humid windless climates, a direct measure of this loss can be obtained by collecting and weighing the rejected material. However, in Canterbury during the lamb-fattening period in summer, damaged or dropped rape cannot be recovered, as it is rapidly dehydrated and often blown away.

The D.M. wastage in grazing averaged 17.1% with a range from 8.0% in the block with the lowest yield to 23.9% in the block with the highest yield. The mean wastage of O.M. for the four blocks was 19.6% with a range of from 13.8% to 23.0%. There was an appreciable difference in ash content between the faeces of the grazing wethers, and of the wethers fed ungrazed rape in digestibility experiments, and it appears that the grazing wethers ingest measurable quantities of soil. Consequently the estimated wastage of D.M. in grazing was probably lower than that of O.M. Furthermore this soil passes to the faeces in quantities in excess of the consumption/excretion ratio established in the digestibility experiments, resulting in over-estimation of rape forage intake and also in underestimation of grazing wastage. The mean grazing wastage of S.E. of blocks II and III was 24.9% as against 21.9% for D.M. and 23.6% for O.M. The wastages of C.P. and E.Ex. were much higher, and that of C.F. was much lower than that of N.F.Ex. which was similar to that of O.M. This implies either that the plant parts containing more C.P. and E.Ex. and less C.F. were wasted in greater quantity, or that E.Ex. was lost as volatile sulphur oils from tissue cut by biting and trampling. The excessive losses of C.P. and E.Ex., which are at a high concentration in the leaf, and of C.F., which is at a high level in the stem, are understandable because wastage occurs by dirtying or destroying dropped and prostrate leaves. The excessive loss of C.P. and of E.Ex. is an unfortunate consequence of grazing, at least with rape. Because of these losses and the comparative indestructibility of C.F. the rape rejected was of very different chemical composition, and food value, from that offered (Table 2). Both D.M., due to ingestion of soil and O.M., because of the changed chemical composition, were less reliable bases of determining grazing wastage than were units of S.E.

Wastage in Digestion

Records of the apparent digestibility coefficients of rape in New Zealand (Lancaster and Adams 1943; Allison and Thurston 1952) and elsewhere (Wood and Woodman 1930) are available. In the first paper of this series the apparent digestibility coefficients of selected rape were

TABLE 2. COMPOSITION OF RAPE IN BLOCKS II AND III

Block No.	Constituent	Composition as Percentage of Organic matter				
		Offered for Eating	Stubble	Apparently Consumed	Consumed	Grazing Wastage
II	Crude protein	11.4	11.1	11.5	9.3	16.6
	Crude fibre	13.2	15.5	12.5	15.2	6.3
	Ether extract	2.3	1.3	2.6	1.1	6.1
	Nitrogen-free extract	73.1	72.1	73.4	74.4	71.0
	Starch equivalents	83.3	82.0	84.1	82.9	
III	Crude protein	12.6	10.5	12.9	9.8	20.8
	Crude fibre	13.1	17.3	12.5	16.9	0.9
	Ether extract	2.2	1.3	2.3	1.6	4.3
	Nitrogen-free extract	72.2	70.9	72.3	71.7	74.0
	Starch equivalents	83.2	81.1	84.0	81.2	

shown to be intermediate between those of ungrazed and grazed rape and similar to reported results for New Zealand rape, but different from results reported on English rape. The minor differences in digestibility between grazed and ungrazed rape in this experiment are reflected in the digestion wastage of Table 1. The mean wastage of D.M. in digestion was 9.6% (15% of intake), while that of O.M. was 6.6% (10% of intake). This wastage is lower than would be expected from the above reports for the reason that only about 65% of the rape offered for eating was consumed and hence subject to digestion wastage.

Total Wastage

The degree of utilisation of the rape offered for eating is shown in Table 1. The mean wastage of D.M. was 44.3% and of O.M. 43.8%. The wastage of S.E. of blocks II and III averaged 42.8%. With good grazing management, normal weather, and average yields, only about 56% of the rape offered for eating was used for growth and maintenance of wethers. It is clear from the compositions of stubble and material wasted in grazing (Table 2) that their combined value is better than that of the rape consumed, and the uneaten material would consequently be capable of fattening sheep. Further, the pattern of differential wastage of proximate constituents is consistent between blocks. As compared with a mean O.M. wastage of 47.7% in blocks II and III, that of E.Ex. was 95.1%, of C.P. 66.9%, and of C.F. 37.7%. The wastage of 44.8% of N.F.Ex. was similar to that of O.M. because it comprised the bulk of the material in both leaf and stem of rape and was little affected by the differential wastage.

Relationship between Yield, Intensity of Grazing, and Wastage of Rape

It is suggested from field observation that the highest wastage of forage occurs either in high-yielding crops or where stocking intensity is highest. The validity of these observations was tested on the figures given in Table 3.

TABLE 3. RELATIONSHIP BETWEEN YIELD, GRAZING INTENSITY, GRAZING WASTAGE, AND OTHER WASTAGE

		Block I	Block II	Block III	Block IV	Correlation Coefficients
A	Yield (lb) O.M. per acre	3367	4347	4067	1849	A & C
	Standard error	±94	±154	±95	±57	0.976*
B	Grazing intensity—lb OM of stubble residues left	693	1024	523	249	A & D 0.875
C	Grazing wastage—lb O.M./acre	586	1001	986	255	B & C 0.721
D	Other wastage—lb O.M./acre (stubble and undigested)	918	1259	784	393	

* Significant at the 5% probability level.

The correlation cofficient of 0.976 between yield and grazing wastage was significant at the 5% probability level, ignoring the complication that the wastage was part of the yield. However the data in Table 3 suggest a curvilinear rather than a linear relationship and the hypothesis that if yields are doubled the grazing wastage is quadrupled was tested. In the equation derived from the regression of log W on log Y,

$$W = aY^b,$$

where W = grazing wastage, Y = yield and a and b are constants, the value of b would equal two if there was a perfect quadratic relationship. For the measurements recorded in Table 3, b was found to be 1.635, and did not differ significantly from two. Though the data are inadequate there is support for the above hypothesis, which is also compatible with field observations of the wastage of rape in grazing, and may be true for other forages, provided the feed available at only one grazing is being considered, and yields are within the normal range. Though the high correlation coefficient of 0.875 between yield and other sources of wastage is suggestive, it is not statistically significant. There is, however, a sound physical basis for a significant relationship, as high-yielding rape crops have lower leaf/stem ratios and so could be expected to have higher stubble residues. Similarly, while the correlation coefficient of 0.721 between grazing intensity and grazing wastage is not significant, it is to be expected that severe grazing, as indicated by lower stubble residues, should be accompanied by a lower grazing wastage. Factors such as methods of grazing, climatic conditions, species, growth habit, stage of growth, and row spacing must also influence wastage. In this experiment it was not possible to examine these.

Possible Sources of Error and their Importance

The measurements recorded in Table 1 were subject to several types of error.

It is known from the accuracy of chemical determinations that errors in these are not likely to affect results in view of the comparatively large differences found. Furthermore the variability of the samples for yield estimates were such that the coefficients of variability of dry-matter weights from the different blocks ranged from 7.5 to 10.3 before grazing, 11.1 to 38.4 after grazing and from 4.9 to 11.7 in the ungrazed blocks, so the sampling errors were relatively small. It is clear from the low standard errors of the digestibility determinations, recorded in the first paper of this series, that these could be only a minor source of error, provided the nature of the feed actually eaten by the grazing wethers did not differ materially from that fed in digest pens, and provided the mean efficiency of digestion of the four wethers in the digestibility experiments was similar to the mean of the sixteen used for grazing. In any event, as shown by Schneider and Lucas (1950) errors due to differences in digestion between sheep are likely to be less than those due to inadequacies of technique or to differences between batches of food. There were only small differences in digestibility of organic matter and of proximate constituents of ungrazed and selected rape, so only small errors were incurred in employing consumption/excretion ratios determined in digestibility experiments, for the purpose of computing the intake of grazing wethers (Lofgreen and Meyer 1956; Greenall 1958). The estimate of total wastage of E.Ex. was higher than expected and may be an exception to this. There is no reason to doubt this estimate provided the assumption of similar digestibilities of E.Ex. for penned and free-grazing wethers is valid. There is, however, a suggestion from the different levels of E.Ex. in the faeces of the penned and the free-grazing wethers that the latter digested E.Ex. more. If so the total wastage of E.Ex. was reduced by this increased utilisation, and by the increased ingestion of E.Ex. calculated from the altered E.Ex. consumption/excretion ratio. It has, however, been established by Blaxter and Graham (1955), Raymond *et al.* (1955), and others, that intake level affects digestibility: the higher the intake the lower the digestibility. The mean intake of the four wethers in digestibility experiments was lower than that of the grazing wethers, whose efficiency of digestion would consequently be lower. The effect would be to overestimate their intake and so underestimate grazing wastage, but the effect would be small and would lead to a conservative estimate of wastage.

As shown by Poijarvi (1952) and more recently by Lambourne (1957) 70% to 80% of the undigested residues from feed eaten is excreted within 24 hours. Though the wethers were starved on gratings for 24 hours before grazing a block, deviation from this rate of passage would result in some error in calculating intake from each block. However, as reported by Meyer *et al.* (1956), periods of even less than 24 hours are sometimes used for this purpose. The total collection of faeces has entirely eliminated fouling of rape from this source, and so has reduced grazing wastage.

DISCUSSION

No reports of the kind and degree of wastage of crop forages during grazing with sheep, or of the factors involved, could be found in the literature, so that the results presented can be compared only with results from investigations on pasture forage. In this experiment about 65% of the D.M. and 63% of the O.M. of the rape offered were consumed. Meyer *et al.* (1956) reported that steers free-grazing pasture utilised 52% of that which could be used by cutting and feeding. The difference was attribute to waste by trampling and in stubble. Peterson *et al.* (1956), recorded for steers rotationally and strip grazing lucerne a utilisation of 71–76%, attributing wastage to loss by leaf drop, fouling, and trampling. This wastage of from 24–29% appears to represent wastage in grazing only, and may be compared with the 17% for rape. The stubble residues following rotational grazing were 50% higher than for strip grazing, which illustrates the effect of methods of management on utilisation.

The amount of rape stubble wasted depended on grazing management. Farmers aim to fatten lambs quickly by maintaining high intake. The decision to discontinue the grazing of a block is dependent on the experience of the farmer, and on the behaviour of the lambs, though the amount and condition of the stubble are also considered. The estimate of stubble wastage in this experiment is affected by the initial decision to manage the wethers according to the best lamb-fattening practice, and is applicable only to such practice. However, the stubble left by lambs, if not capable of providing a fattening ration will at least provide a maintenance ration for store animals. It was shown above that wastage is probably related to yield of rape as well as to intensity of grazing.

From the limited data available it would appear that the efficiency of utilisation of total D.M. by sheep grazing rape is similar to that of pasture and lucerne. That the loss of O.M. may be greater than that of dry matter because of ingested soil is inferred from the experiments of Garrigus and Rusk (1936) and of Lofgreen and Meyer (1956), and is shown in these experiments with rape to be a factor of methodological significance. No record of comparable measures of wastage of S.E., or of differential wastage of proximate constituents could be found in the literature. However there is an implicit acceptance of the occurrence of some degree of selective grazing, or of differential wastage of plant parts of different composition. As has been reported by Ahlgreen (1947), Peterson *et al.* (1956), and others, yields determined by clipping methods frequently cannot be reconciled with those obtained by animal methods. This must obviously be so with grazing of rape where a grazing wastage of S.E. of up to 26% has been shown to occur.

Several factors of crop and animal management are likely to influence the extent of wastage. Some of these factors are amenable to manipulation, so as to achieve better utilisation. Three levels of stocking intensity, conforming to the established practice of set stocking, block grazing, and strip grazing, might profitably be compared, keeping in mind, however, the effect of disturbance on the weight gain of lambs, in particular. The effects of variation in row spacing and seeding rates, aimed at providing the bulk of the forage in a position inaccessible to trampling and fouling, could be examined. At present prices of lamb meat, and with mechanisation at a high level, the almost complete utilisation that should accompany cutting with a silorator and dry-lot feeding, might profitably be investigated.

Lastly, it is clear that forage yields obtained by a cutting technique are likely to provide a poor basis of prediction of animal production from grazing.

ACKNOWLEDGEMENTS

I gratefully acknowledge the technical assistance of Mr. J. Webb, and the services of the Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for the statistical analyses, and of the Biochemistry Section of this Division for chemical analyses.

REFERENCES

AHLGREEN, H. L. 1947: *J. Amer. Soc. Agron. 39:* 240-59.
ALLISON, R. M.; THURSTON, W. G. 1952: *N.Z. J. Sci. Tech. A34:* 341-6.
ANON. 1952: Report on Pasture and Range Research Techniques. 1952: *Agron. J. 44:* 39-49.
BLAXTER, K. L.; GRAHAM, N. MCG. 1955: *J. agric. Sci. 46:* 292-306.
BRISSON, J. G.; ANGUS, W. J.; SYLVESTRE, P. E. 1954: *Canad. J. agric. Sci. 34:* 528-32.
BROWN, DOROTHY 1954: *Bur. Past., Aberystw. 42:* 223 pp.
GARRIGUS, W. P. 1935: *Proc. Amer. Soc. Anim. Prod. 27:* 66-9.
GARRIGUS, W. P.; RUSK, H. P. 1936: *Ibid. 28:* 75-82.
GREENALL, A. F. 1958: *N.Z. J. agric. Res. 1:* 569-81.
KANE, E. A.; ELY, R. E.; JACOBSON, W. C.; MOORE, L. A. 1953: *J. Dairy Sci. 36:* 325-33.
LAMBOURNE, L. J. 1957: *J. agric. Sci. 48:* 415-25.
LANCASTER, R. J. 1954: *N.Z. J. Sci. Tech. A36:* 15-40.
LANCASTER, R. J.; ADAMS, A. F. R. 1943: *N.Z. J. Sci. Tech. A25:* 131-6.
LOFGREEN, G. P.; MEYER, J. H. 1956: *J. Dairy Sci. 39:* 268-73.
MEYER, J. H.; LOFGREEN, G. P.; ITTNER, N. R. 1956: *J. Anim. Sci. 15:* 64-75.
PETERSON, M. L.; LOFGREEN, G. P.; MEYER, J. H. 1956: *Agron. J. 48:* 560-3.
POIJARVI, I. 1952: *Maataloust. Aikakausk. 24:* 1.
RAYMOND, W. E.; HARRIS, C. E.; KEMP, C. D. 1955: *J. Brit. Grassl. Soc. 10:* 19-26.
RAYMOND, W. E.; MINSON, D. J.; HARRIS, C. E. 1956: *Proc. 7th int. Grassl. Congr.:* 123-32.
REID, J. T.; WOOLFOLK, P. G.; RICHARDS, C. R.; KAUFMANN, R. W.; LOOSLI, J. K.; TURK, K. L.; MILLER, J. I.; BLASER, R. E. 1950: *J. Dairy Sci. 33:* 60-72.
SCHNEIDER, B. H.; LUCAS, H. L. 1950: *J. Anim. Sci. 9:* 504-12.
VAN DER KLEY, F. K. 1956: *Netherlands J. agric. Sci. 4:* 197-204.
WOOD, T. B.; WOODMAN, H. E. 1930: *Minist. Agric. Fish., Lond. Misc. Publ. 32,* 61 pp.
WOODMAN, H. E.; EVANS, R. E.; EDIN, A. 1937: *J. agric. Sci. 27:* 212-23.

EFFECT ON HONEY BEES OF TOXAPHENE AND STROBANE APPLIED TO WHITE CLOVER PASTURE

By T. Palmer-Jones†, I. W. Forster*, and L. J. S. Line*

(*Received for publication, 21 April 1958*)

Summary

Toxaphene was sprayed and dusted over 12 acres of white clover pasture at the rate of 5 lb of actual toxaphene per acre.

Strobane was sprayed over 8 acres of white clover pasture at the rate of 5 lb of actual strobane per acre.

All applications were made in the early morning when no bees were flying.

Bees from nearby experimental apiaries collected nectar extensively from the clover flowers in both experimental areas soon after all these applications; pollen was also collected.

There was no evidence of bee mortality or adverse effect upon brood after the toxaphene applications. A slight mortality in field bees was apparent after the strobane application but no dead bees were brought out of the hives and brood was unaffected.

It is considered that toxaphene causes no bee mortality when applied to clover pasture. Strobane causes only negligible losses of bees and is safe to use. These conclusions apply only when toxaphene and strobane are applied at a time when bees are not flying.

It is unlikely that toxaphene would cause bee mortality if put into general use on crops; strobane can safely be used on clover pasture, but should be applied with caution elsewhere.

INTRODUCTION

White-clover pasture (*Trifolium repens*) throughout New Zealand is subject to attack from the clover case-bearer moth (*Coleophora spissicornis* and *C. alcyonipennella*?). The caterpillars of these moths feed on the developing seed heads to such an extent that there have been cases where seed growers have lost 80% of the crop. It is estimated that approximately 10% of clover seed is destroyed by the moth and in recent years, depending on the extent of seed production, the national loss has been from £60,000 to £105,000 yearly. Biological control is being investigated by the Department of Scientific and Industrial Research, but this is necessarily a long-term project and it was felt that the possibility of immediate control by agricultural chemicals should be investigated.

It is considered that one of the most likely times at which the clover case-bearer would respond to control by pesticides is in the spring and summer, which is a period when the infested clover pasture is also attractive to honey bees. Consequently it was decided that for work at this time of the year the first step should be for the Department

---

† Animal Research Station, Department of Agriculture, Wallaceville.
* Horticulture Division, Department of Agriculture, Wellington.

N.Z. J. agric. Res. 1: 694–706.

of Agriculture to carry out trials designed to find pesticides which could be applied without harming honey bees. Then the Department of Scientific and Industrial Research would test the effect of these compounds upon the clover case-bearer.

Accordingly, laboratory tests designed by Palmer-Jones (1958) to study the effect upon honey bees of pesticides were used to screen a range of pesticides. Results showed that toxaphene and strobane were the least likely to cause bee mortality in field applications.

Toxaphene has the empirical formula $C_{10}H_{10}Cl_8$ and is a chlorinated camphene. It has been reported by other workers that it can be applied to flowering plants with relative safety to bees (Stute 1954; Weaver 1953). Strobane is the chlorination product of a mixture of terpene hydrocarbons and has a chlorine content of approximately 66%. Field trials designed to test its effect on honey bees have not been reported. The literature on these pesticides has been reviewed recently by Palmer-Jones (1958).

The following field trials resembling those carried out previously by Palmer-Jones *et al.* (1954, 1957) were designed to find what effect toxaphene and strobane would have upon honey bees when applied at maximum rates to white-clover pasture.

EXPERIMENTAL AREAS

Two experimental areas of 12 and 8 acres were used. They were situated on the farm of Mr. D. W. Van Asch at Te Awanga, Hawke's Bay, approximately 6 miles in a straight line from Hastings (Figs. 1 and 2).

The toxaphene sprays and dust were applied to the 12 acre area (290.4 x 200 yd) while the strobane spray was applied to the 8 acre area (242 x 160 yd).

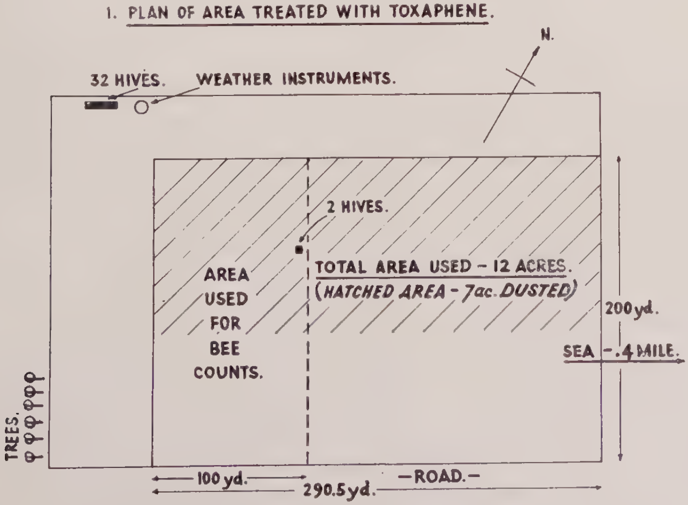

1. PLAN OF AREA TREATED WITH TOXAPHENE.
N.
32 HIVES.
WEATHER INSTRUMENTS.
2 HIVES.
AREA USED FOR BEE COUNTS.
TOTAL AREA USED - 12 ACRES.
(HATCHED AREA - 7ac. DUSTED)
200yd.
SEA - .4 MILE.
TREES.
100 yd.
290.5yd.
-ROAD.-

Fig. 1.—Area treated with toxaphene.

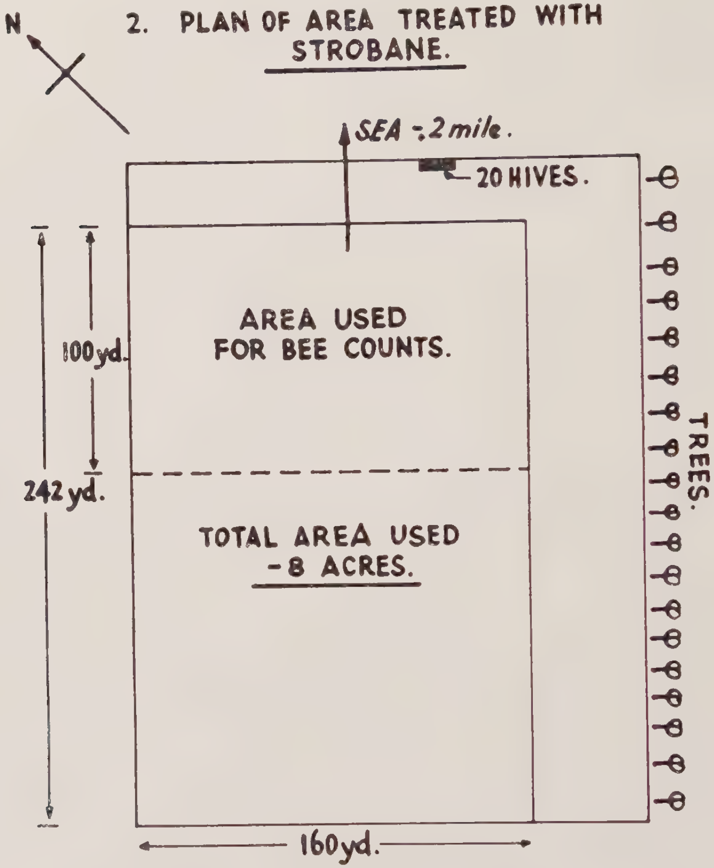

N
2. PLAN OF AREA TREATED WITH STROBANE.
SEA -.2 mile.
20 HIVES.
AREA USED FOR BEE COUNTS.
100 yd.
242 yd.
TOTAL AREA USED -8 ACRES.
TREES.
160 yd.

Fig. 2.—Area treated with strobane.

Both areas were marked out in large fields of white clover (*Trifolium repens*) pasture with a fairly heavy overgrowth of perennial ryegrass (*Lolium perenne*). Other floral species were almost entirely absent. The fields had been closed to stock shortly before the experiment commenced and the pasture was covered with clover flowers attractive to bees at the time of the applications.

The clover flowers in the two fields were the main nectar source in the immediate vicinity of the experimental apiaries. Bees were observed to collect pollen from clover flowers in both fields and also collected it from flowers in neighbouring gardens.

Thirty-four hives of bees were moved on 17 September to the site of the 12-acre area. Two hives were placed well inside the 12 acres (Fig. 1). On 11 December twenty hives of bees were moved to the site of the 8-acre area.

The hives on both sites were of a strength which would be attained by an experienced commercial beekeeper at the time and in the location of the experiment. They were Langstroths averaging slightly less than four storeys and with a brood area of approximately six frames (both sides).

The layer of grass in front of all hives was removed to expose a bare surface on which dead bees would be easily seen.

EXPERIMENTAL PROCEDURE

Weather Observations

A thermo-hygrograph was installed in a Stevenson screen and daily readings of wind velocity were made with a portable anemometer. Ground thermometers (2, 4, and 8 inch) were read daily. Rainfall was measured and cloud and general weather conditions noted (Fig. 3 and Table 1) During the course of the experiment, recording ground thermometers showed a variation of 56° to 78°F at 4 inch, and 60° to 70°F at 8 inch.

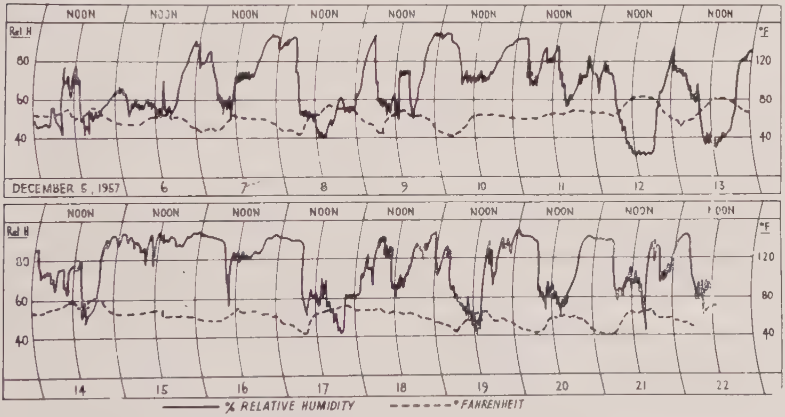


Fig. 3.—Relative humidity and temperature.

Weather observations were made on the 12-acre site. However, with the exception of wind velocities, results would be much the same on the other site as it was distant only three-quarters of a mile; wind velocities would be less there because it was more sheltered.

Application of Toxaphene and Strobane

Toxaphene Spray.—Toxaphene emulsion (50% w/v) was mixed with water to give a concentration of 5%. Ten gallons of this dilution, containing 5 lb of toxaphene, was applied per acre from the air to the 12-acre site.

Spraying finished by 6 a.m. on 8 December. At this time all the bees were within their hives and so none were exposed directly to the spray. A Tiger Moth plane with standard low-volume boom spray rig was used to apply the spray from a height of 3 ft (nozzle to ground). A dead calm prevailed and conditions were ideal for application, no

TABLE 1. WEATHER CONDITIONS AND GENERAL INFORMATION

Date December 1957	Rainfall 24 hour 9 a.m.	Wind		Ground temperature 9 a.m.			Weather	General
		Time of reading	Velocity (m.p.h.) and direction	2″	4″	8″		
4	—	9 a.m. 9.30 a.m. 11.45 a.m. 2.15 p.m. 4 p.m.	5.6 S.E. 6.3 S.E. 7.7 E. 9.3 E. 6.4 E.	68	59	60	Cloud 1/10, complete haze noon, late afternoon cloud 0/10.	Bees active all day.
5		9.30 a.m. 2.15 p.m.	11.9 W. 7 W.	70	65	64	Cloud 10/10 morning. Bright periods afternoon with gusts of wind and light rain.	No bee activity morning.
6	Trace	9 a.m. 10 a.m. 2 p.m.	8.9 S.W. 7 W. 11.3 S.	71	61	62	Cloud 9/10 morning. Sun afternoon with 5/10 cloud.	Bees active all day.
7	—	9 a.m. 10.30 a.m. 2.15 p.m.	3.7 W. 3.7 N.E. 7.5 N.E.	71	61	62	Cloud 5/10 morning. Intermittent sunshine all day.	Bees active all day.
8	—	6.30 a.m. 9.30 a.m. 12 noon 2 p.m.	5.6 S.W. 8.7 S.W. 18.2 W. 18.9 W.	64†	62†	62.5†	Cloud 1/10 morning. 9/10 3 p.m. Strong winds all day. Heavy dew. († Read 9.45 a.m.)	Toxaphene spray applied by 6 a.m. No bee activity morning, some afternoon.
9	—	9 a.m. 10.30 a.m. 4 p.m.	2.2 S.W. 8.8 S.W. 9.4 N.E.	71	60.5	61.5	Cloud 3/10 all day.	Bees active all day.
10	—	9 a.m. 11 a.m. 2 p.m. 4 p.m.	3.6 N.E. 7.6 N.E. 8.8 E. 9.2 E.	71.5	61.5	62.5	Cloud dropped from 6/10 morning to 3/10 afternoon. Light dew.	Toxaphene spray applied by 5.15 a.m. Bees active all day.

	—	9.30 a.m. 2 p.m.	6.5 8.5	N. N.W.	70	65.5	65	Cloud 10/10 all day. Showers in morning.	No bee activity all day.
2	.04	10 a.m. 11 a.m. 3 p.m.	11.9 14 16.7	W. W. W.	82.5†	66†	63†	Cloud 1/10 all day. Bright sunshine. († Read 10 a.m.)	Very little bee activity owing to high wind.
3	—	9 a.m. 11 a.m. 2.30 p.m.	7.8 4.9 8.4	N.W. N.W. W.	79	66	65	Cloud increasing from 1/10 to 4/10.	Bees very active all day.
14	—	9 a.m. 2 p.m.	14.9 11.3	N.W. N.	77	69	67	Cloud increasing from 1/10 to 4/10. Sun.	Bees active all day.
15	.19	9.45 a.m. 2.30 p.m.	5.6 5.8	N.W. N.E.	69†	69†	69†	Cloud 9/10 with rain at intervals. († Read 9.45 a.m.)	No bee activity all day.
16	.38	9 a.m. 12.30 p.m.* 4.30 p.m.*	2.3 10.4 8	N. E. E.	75	63	64	Cloud 2/10 all day. Sun. Heavy dew.	Strobane spray applied by 7.45 a.m. Bees very active all day.
17	—	9 a.m. 2.30 p.m.*	4.7 11	N. E.	72	62	65	Cloud 0/10 all day.	Bees very active all day.
18	.11	9 a.m.	0.7	N.	68	68	68	Cloud 9/10 morning. Rain early morning.	No bee activity.
19	.09	10 a.m.	12.2	S.W.	73.5†	61†	62†	Cloud 3/10 with sun morning. Cloud 9/10 late afternoon with rain. († Read 10 a.m.)	Bees active all day.
20	.16	12.30 p.m.	9.4	S.E.	79.5†	65.5†	62†	Cloud 5/10 with intermittent sun. Heavy dew. († Read 12.30 p.m.)	Toxaphene dust applied by 9.30 a.m. Bees active all day.
21	—	9 a.m.	5.1	N.W.	70	58.5	61	Haze, bright sun at intervals morning, then overcast.	Bees very active all day.
22	.11	10 a.m.	6	E.	78†	63†	62†	Heat haze, then bright sun with 2/10 cloud. († Read 9.45 a.m.)	Bees very active all day.

(* Readings made on area treated with Strobane) spray drift occurring. At the time of spraying the pasture was wet with a heavy dew.

Soon after spraying, the wind rose and became so strong that there was no bee activity in the morning and little in the afternoon. However, bees were active throughout the next day.

Because of lack of bee activity on the day of spraying a toxaphene spray of similar strength was applied again from the air in the same way on 10 December under ideal conditions. Spraying finished at 5.15 a.m., before bee activity commenced. At the time of application there was a light dew. Bees were active all day after the spraying.

Toxaphene Dust. Fifty pounds per acre of 10% toxaphene dust (5 lb of actual toxaphene per acre) was applied to 7 acres of the 12-acre site. It had been intended to apply the dust by air to the whole 12 acres but no suitably equipped aeroplane was available. Two of us finished the operation by 9.30 a.m. on 20 December just before bees commenced visiting the pasture which was wet with a heavy dew. Bees were active on the days following application.

Strobane Spray. Strobane emulsion (40% w/v) was mixed with water to give a concentration of 5%. Ten gallons of this dilution, containing 5 lb of strobane, was applied per acre to the 8-acre site.

The spray was applied from a truck equipped with a standard low-volume boom spray rig for hormone application. Weather conditions were ideal. Spraying ceased at 7.45 a.m. on 16 December. The pasture was covered with a heavy dew at this time and bees had not yet commenced to visit it. Bees were on the pasture in large numbers for two days following application.

Counts of Field Bees

Counts of field bees were made in representative marked sections of both experimental areas (Figs. 1 and 2) as described by Palmer-Jones *et al.* (1957). The total bee count obtained for 400 sq. yards was multiplied by 12.1 to give the bee densities per acre plotted in Figs. 4 and 5.

Counts of Clover Flowers

Each group of results shown in Fig. 6 represents a total of twenty counts of clover flowers made within a wire gauge one foot square. The procedure followed was for one of us to walk at random across an experimental area, place the centre of one side of the wire gauge against the toe of his forward shoe at the completion of each ten paces, and then count all the flower heads enclosed by the gauge. Twenty counts were made, flower heads being classed as flowering or seeded. Seeded heads were considered to be those in which all the florets were folded down.

It is apparent from Fig. 6 that the number of clover heads in flower on the experimental areas remained fairly constant during the course of the experiment. But the total number of clover flowers tended to increase, particularly on the 8-acre site, because of an increase in the number of seeded flower heads.

Identification of Bees on Crop

No commercial or private apiary was within effective bee range of the 12-acre experimental site; one apiary of three weak hives close to the 8-acre site could be disregarded. Some bees from the experimental hives on the 12-acre site visited the pasture on the 8-acre site but this would not affect the issue as the apiary from which these came was under continuous observation.

RESULTS

Counts of Field Bees

Area Treated with Toxaphene. Bees visited the area in large numbers on 4, 6, and 7 December (Fig. 4 and Table 1). Bee activity was nil in the morning and low in the afternoon of the 5th owing to high winds. The toxaphene spray was applied by 6 a.m. on the 8th. On this day there was no bee activity in the morning and little in the afternoon owing to strong winds. But bee activity was high on the 9th and also on the 10th when toxaphene spray was again applied. Spraying was over by 5.15 a.m., before bees visited the pasture. There was no bee activity on the 11th when 4 points of rain fell. Winds hindered bee activity on the 12th but they were active on the 13th and 14th.

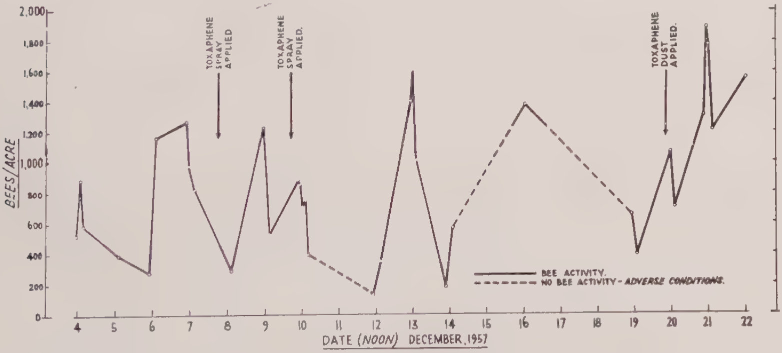


Fig. 4.—Bee activity on toxaphene area.

Toxaphene dust was applied on the 20th before bees visited the pasture. Bees were very active on this and the two following days. Eleven points of rain fell on the night of the 21st and must have washed off some of the dust.

Area Treated with Strobane. Bees were active all day on this during 13 and 14 December, but owing to rain were not flying on the 15th. The spray was applied under ideal conditions by 7.45 a.m. on the 16th before bees were on the pasture. Bees visited the area all day on the 16th and 17th. There was no bee activity on the 18th but bees were observed to be active from the 19th until the experiment closed on the 22nd (Fig. 5).

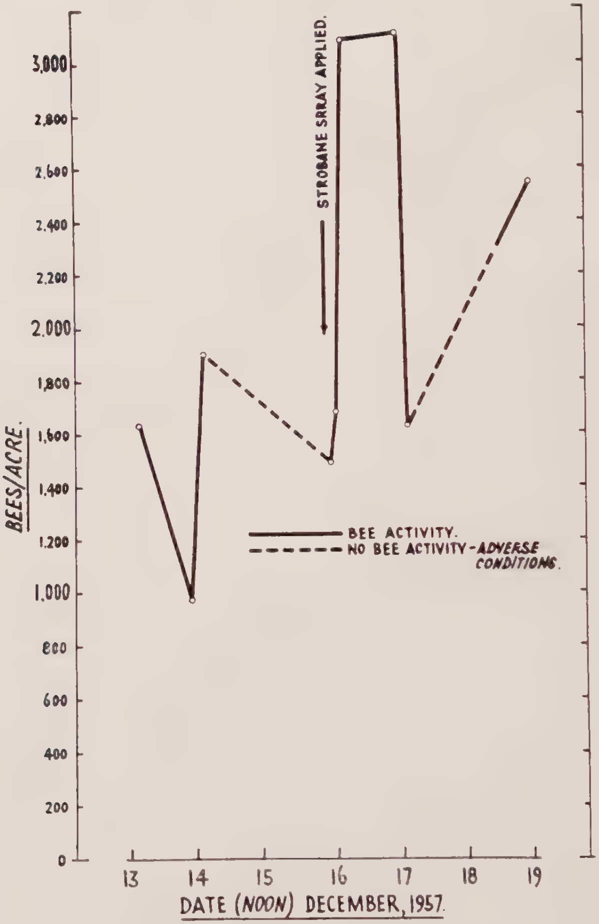


Fig. 5.—Bee activity on strobane area.

Behaviour of Bees on Crop

The strong winds which persisted during the course of the experiment often made it difficult for bees to alight on the clover flower heads, blowing them against the stalks of the ryegrass. They thus came more in contact with the sprayed or dusted pasture than would be usual. However, the behaviour of the bees was perfectly normal after the toxaphene and strobane applications and there was no evidence of repellency.

Very high bee counts were obtained for two days following the strobane application. There was no evidence that the toxaphene sprays and dust depressed bee counts and bee activity appeared as great inside the treated area as outside.

It is considered that toxaphene and strobane are not repellent to bees.

Effect of Insecticides upon Field Bees

As in former field experiments carried out by Palmer-Jones *et al.* (1954, 1957) the toxic action of strobane and toxaphene was studied by caging field bees and observing them over a 48-hour period. These were picked off clover flowers in the areas treated with insecticide and placed in cages supplied with feeders holding sugar syrup: the cages were kept in a 30°C incubator. Control bees showed negligible mortality after 48 hours when held under such conditions.

Toxaphene. Twenty-nine field bees collected during the afternoon of the day when the first toxaphene spray was applied showed no undue mortality. Fifty-five bees collected during the morning and afternoon of the following day showed a mortality of only two bees.

On the day the second toxaphene spray was applied, forty bees collected in the morning, and twenty in the afternoon, showed no more than average mortality.

Bees collected on the day the toxaphene dust was applied and over the following day were not affected unduly.

Strobane. Fifty-one bees collected at intervals during the day strobane was applied showed a mortality of 29% which is about 25% more than the normal mortality of approximately 4%. Bees collected on the following day showed no undue mortality.

Effect of Insecticides upon Colonies

The hives were examined during the course of the experiment and a fortnight later, being finally kept under observation for some months after its termination.

The toxaphene and strobane applications had no adverse effect upon brood or hive activity. No unusual number of dead bees was brought out of the hives during the course of the experiment. The two hives placed within the twelve-acre area sprayed and dusted with toxaphene were unaffected.

Bumble Bees

Practically no bumble bees were observed during the course of the experiment and the effect of the insecticides upon them could not be determined. The few specimens seen were *Bombus terrestris.*

Protection of Observers against Pesticides

Rubber gloves and overalls were worn when handling the toxaphene and strobane solutions. In addition eye shields, gumboots, and rubber aprons were worn when the toxaphene dust was applied by hand. No protective clothing was considered necessary when making observations on the sprayed or dusted pasture. For some time after application toxaphene could be smelt on the pasture, the not unpleasant smell resembling that of a disinfectant. Although we all spent much time on the pasture immediately after the toxaphene applications the only ill effect was sometimes a slight nasal congestion on the day of application, We were completely unaffected by strobane which had only a slight smell.

Discussion

Bees are usually most susceptible to the action of a pesticide on the day it is applied. Consequently the aim in a field experiment such as this is to apply the pesticide on a day when flowers on the treated area are visited by many field bees.

When bee activity was at its height, after the second application of toxaphene spray, bee counts showed that over 12,000 bees at a time were on the treated area. Seven thousand bees at a time, and sometimes more, were on the smaller area dusted with toxaphene.

If we consider both applications of toxaphene sprays together, it is apparent that they resulted in the exposure of large numbers of field bees to at least 5 lb of toxaphene per acre on the day of spraying and the two following days. The slight rain which then fell would probably wash very little toxaphene off the pasture so exposure could be said to extend over a week on only one day of which there was no bee activity.

Many field bees were also exposed for two days to toxaphene dust applied at the rate of 5 lb toxaphene per acre.

There was no evidence that the toxaphene sprays and dust caused mortality in field bees. No dead bees were brought out of the experimental hives and young bees and brood were unaffected, although pollen from sprayed and dusted clover flowers was collected.

The toxaphene exerted no repellency so there was no reduction in the number of field bees visiting the experimental area after the applications.

The strobane spray was applied at the rate of 5 lb strobane per acre on a day when conditions were so favourable that one count showed over 24,000 bees on the 8-acre area sprayed. Conditions were equally favourable on the following day.

Field bees picked off the crop and caged on the day strobane was applied showed some mortality but not these collected on the following day. No dead bees were brought out of the hives and young bees and brood were unaffected although pollen was collected from the treated area. The effect on the apiary was considered to be negligible.

If the number of clover heads in flower on the 12-acre area is taken as averaging 150 per 20 sq. ft, this gives a figure of 326,700 flowers per acre (Fig. 6). Peak bee densities reached 1,200 bees per acre, equivalent to one bee per 272 flowers.

On the 8-acre site, clover heads in flower averaged 200 per 20 sq. ft and peak bee densities reached 3,000 per acre, equivalent to one bee per 145 flowers.

In a recent publication Palmer-Jones (1958) predicted, as a result of laboratory tests, that field applications of toxaphene and strobane

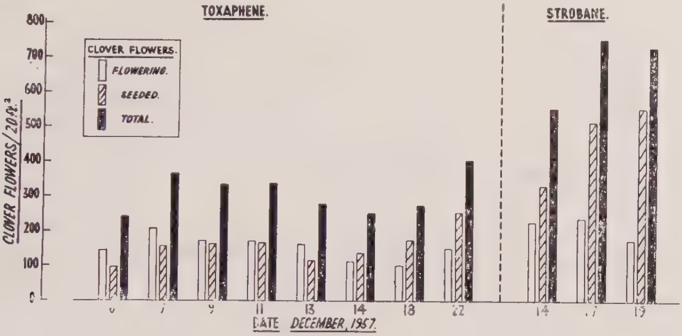


Fig. 6.—Clover flowers per 20 sq. ft. in treated areas.

would be expected to cause little mortality if made when bees were not active. The experiment shows the value of these laboratory tests.

Both toxaphene and strobane will be used by the Department of Scientific and Industrial Research in trials against the clover case-bearer moth. These pesticides are also likely to prove of value in controlling pests on crops which are attractive to bees.

CONCLUSIONS

It is considered that strobane spray and toxaphene spray and dust, if applied so that they do not come in direct contact with bees, can be safely used on white-clover pasture at the maximum rate of 5 lb per acre.

Conditions are frequently such in the early spring that almost all the field bees in an apiary may be concentrated on a few acres of a crop. It is unlikely that mortality would occur if such a crop were sprayed or dusted with toxaphene but it is possible that strobane might be dangerous as a slight loss of field bees occurs when it is applied to clover pasture. So in such cases strobane should be used with caution.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Mr. D. W. Van Asch, Te Awanga, near Hastings. Mr. Van Asch made available both trial areas and gave every assistance possible to the extent of applying the strobane spray himself. Messrs. Ivon Watkins, Ltd., New Plymouth, and Messrs. Ivory Spray Chemicals, Ltd., Nelson, generously gave the pesticides used in the trials. Thanks are due to Mr. A. L. Bryant, Photographer, Animal Research Station, Wallaceville, for drawing and photographing the figures. Mr. R. Odinot, Therapeutant Specialist, Horticulture Division, Wellington, supplied information. Dr. W. Cottier gave the figures quoted in the Introduction. We are grateful to Mr. F. Collin, Instructor in Agriculture, Hastings; Mr. G. F. R. Gordon, Apiarist, Hastings, from whom the hives were hired; Mr. R. G. Janson, Hastings, who represented Messrs. Ivory Spray Chemicals; and Messrs. Aerial Agriculture Ltd., Hastings, and their pilot, Mr. R. Snell.

REFERENCES

PALMER-JONES, T. 1958: Laboratory Methods for Measuring the Toxicity of Pesticides to Honey Bees. *N.Z. J. agric. Res. 1:* 290-300.

PALMER-JONES, T.; BARTRUM, F. A.; FORSTER, I. W.; HARRISON, D. L. 1954: Effect on Honey Bees of DDT Plus Superphosphate Applied as a Dust to White Clover Pasture. *N.Z. J. Sci. Tech. A36:* 177-92.

PALMER-JONES, T.; FORSTER, I. W.; GRIFFIN, L. A. M. 1957: Effect on Honey Bees of Metasystox Applied from the Air as a Spray to Chou Moellier. *N.Z. J. Sci. Tech. A38:* 752-69.

STUTE, K. 1954: The Action of Toxaphene Dust on Bees. *Anz. Schädlingsk. 27:* 28.

WEAVER, NEVIN 1953: Toxicity of Insecticides to Honeybees. *Texas agric. Exp. Sta. Progr. Rep. 1554.*

LEAF DEVELOPMENT IN SWARDS OF WHITE CLOVER (*Trifolium repens* L.)

By R. W. BROUGHAM, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 6 June 1958*)

Summary

An experiment is described in which the influence of development and senescence of leaves that were initiated at different stages of regrowth, on the Leaf Area Index (L.A.I.) of white clover swards, was determined.

The total L.A.I. of the swards reached a ceiling value of 5.5 twenty days from defoliation, and thereafter until the end of the experiment only small changes occurred. Of this leaf, 3–3.5 units were fully opened, actively photo-synthesising leaves, and 0.5 units were bud leaves. The remainder were either axillary leaf (0.5 units), or leaf in various stages of senescence (1–1.5 units).

The pattern of sward development was markedly influenced by petiole and lamina development of individual leaves initiated at different stages of growth.

The laminae of leaves initiated after the ceiling L.A.I. was attained were twice the size of the first-formed leaves, and petiole dimensions were 2.5 and 4 times the length and weight respectively. The dimensions of leaves marked on intermediate dates increased progressively to these maximum values.

This pattern of leaf and sward growth is discussed in relation to the light requirements of developing leaves.

The significance of leaf death on the turnover of symbiotically fixed nitrogen is also discussed.

INTRODUCTION

In a previous experiment (Brougham 1958) it was shown that pure stands of white clover growing in this locality, in summer, attained a ceiling Leaf Area Index* of approximately 5.5. It was suggested that a state of equilibrium then existed between death of old leaves and the growth of new leaves. It was also suggested that death of the older leaves was a significant factor in the turnover of symbiotically fixed nitrogen.

The present experiment was designed to determine the life expectancy of white-clover leaves developing in white-clover swards at different stages of growth following defoliation. Because of the dependence of total dry-matter production on the size of the photosynthetic system (Watson 1956), the life expectancy of leaves after the system had reached its maximum, as indicated by a ceiling L.A.I., was of particular interest. By determining the nitrogen content of leaves at different stages of senescence, information was also obtained on the significance of leaf death on the turnover of symbiotically fixed nitrogen.

---

* The ratio of leaf area to ground area occupied and hereafter abbreviated to L.A.I. (Watson 1947).

N.Z. J. agric. Res. 1: 707–718.

It was considered that the results obtained would assist in the interpretation of data obtained from grazing experiments as well as enabling grazing systems to be designed in which excessive losses of leaf tissue are prevented.

EXPERIMENTAL METHODS

The work was carried out at the Palmerston North station of the Grasslands Division, Department of Scientific and Industrial Research, on Manawatu silt loam.

Four established pure stands of New Zealand certified white clover, in plots 10 ft x 8 ft, were defoliated to ½ in. on 6 November 1957. At 5-day intervals from this date, herbage from four randomly sited ½ sq. ft quadrats was harvested. The herbage in each quadrat was cut to the ½ in. level. In the laboratory, the leaves from each sample were separated into the following classes:

Class I.	Leaves with folded laminae.
Class II.	Leaves with laminae opened.
Class III.	Leaves with laminae opened, but showing some yellowing.
Class IV.	Leaves with laminae opened, but showing complete yellowing.
Class V.	Dead leaves, completely brown.
Axillary leaves.	These were of much smaller size.

From each of these sub-samples the laminae were detached from the petioles and the L.A.I. determined by a method previously described (Brougham 1956).

At 6-day intervals from the defoliation date, three separate groups of 300 terminal bud leaves with petioles less than 20 mm in length, were marked with coloured wool, along line transects within the plots. There were seven marking dates, and, at 2-day intervals following each, a number of the marked leaves with petioles intact (10–15 leaves in each replicate), were harvested and removed to the laboratory. For each marking date, this harvesting procedure continued until leaf death occurred.

In the laboratory each group of leaves was divided into the various classes (excluding axillary leaves) and after the laminae were detached from the petioles the following measurements were made:—

(1) Individual petiole length.

(2) Dry weight of the total number of petioles in each class.

(3) Dry weight of the total amount of leaf lamina in each class.

(4) Dry weight of 36 discs (5.5 mm diameter) from a number of leaves in each class.

This last measurement was made so that calculations of the leaf area in each class, and hence the mean leaf area of individual leaves, could be determined.

The final harvest was made on 10 January 1958.

The dried material obtained from the L.A.I. samplings was kept, and at the end of the experiment the total nitrogen in Class V leaves was determined by the Kjeldahl method.

RESULTS

The results presented in Fig. 1 show the L.A.I. of the various classes of leaves and axillary leaves plotted against time. The total L.A.I. with standard errors of estimate are also shown.

Table 1 shows mean petiole length of individual leaves, together with the number of leaves in each class, for the seven marking dates, at each day of sampling.

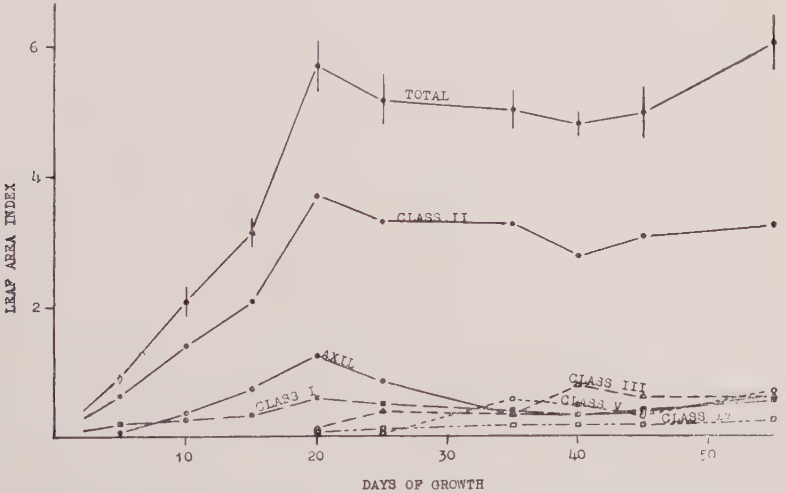


Fig. 1.—L.A.I. of the different classes of leaves at various stages of regrowth. The standard errors of estimate are also shown for total L.A.I. at each sampling.

Because of the large number of determinations made, the data relating petiole weight, area and weight of the individual leaf lamina, and the ratios of lamina to total leaf, to leaf age, are not presented in tabular form. However they are shown for each marking date in Fig. 2.

Polynomials were fitted in order to smooth measurement errors and allow the relationships between leaf dimensions and age to be examined. In all cases, except the lamina area by time curves for the first and second marking dates, no significant improvement was obtained by fitting polynomials of a higher order than the cubic. For the two exceptions the quartic term was significant and for this reason polynomials of this order were fitted. The average standard errors of estimate for each marking date are also shown.

TABLE 1. MEAN PETIOLE LENGTH (millimetres) OF INDIVIDUAL LEAVES FOR THE SEVEN
SHOWN IN

Day of Marking	Leaf Class	Leaf Age (days)								
		2	4	6	8	10	12	14	16	18
1	I	21(45)	26(15)	—	—	—	—	—	—	—
	II	—	33(29)	42(45)	52(45)	68(45)	79(45)	88(45)	101(45)	100(32)
	III	—	—	—	—	—	—	—	—	84(11)
	IV	—	—	—	—	—	—	—	—	—
	V	—	—	—	—	—	—	—	—	—
	Mean	21(45)	31(44)	42(45)	52(45)	68(45)	79(45)	88(45)	101(45)	96(43)
7	I	15(45)	24(17)	—	—	—	—	—	—	—
	II	—	36(28)	69(45)	84(45)	104(45)	113(45)	120(45)	130(45)	135(45)
	III	—	—	—	—	—	—	—	—	—
	IV	—	—	—	—	—	—	—	—	—
	V	—	—	—	—	—	—	—	—	—
	Mean	15(45)	31(45)	69(45)	84(45)	104(45)	113(45)	120(45)	130(45)	135(45)
13	I	22(45)	40(45)	—	—	—	—	—	—	—
	II	—	—	65(45)	101(45)	119(45)	145(44)	175(44)	185(45)	192(44)
	III	—	—	—	—	—	—	—	—	—
	IV	—	—	—	—	—	—	—	—	—
	V	—	—	—	—	—	—	—	—	—
	Mean	22(45)	40(45)	65(45)	101(45)	119(45)	145(44)	175(44)	185(45)	192(44)
19	I	35(45)	66(45)	97(35)	—	—	—	—	—	—
	II	—	—	135(10)	141(44)	180(44)	192(45)	190(44)	220(45)	222(44)
	III	—	—	—	—	—	—	—	—	—
	IV	—	—	—	—	—	—	—	—	—
	V	—	—	—	—	—	—	—	—	—
	Mean	35(45)	66(45)	105(45)	141(44)	180(44)	192(45)	190(44)	220(45)	222(44)
25	I	25(20)	67(20)	87(20)	—	—	—	—	—	—
	II	—	—	—	138(20)	205(19)	199(19)	241(20)	245(20)	251(19)
	III	—	—	—	—	—	—	—	—	—
	IV	—	—	—	—	—	—	—	—	—
	V	—	—	—	—	—	—	—	—	—
	Mean	25(20)	67(20)	87(20)	138(20)	205(19)	199(19)	241(20)	245(20)	251(19)
31	I	—	57(30)	82(29)	—	—	—	—	—	—
	II	—	—	—	139(29)	162(30)	194(30)	223(29)	239(29)	263(20)
	III	—	—	—	—	—	—	—	—	—
	IV	—	—	—	—	—	—	—	—	—
	V	—	—	—	—	—	—	—	—	—
	Mean	—	57(30)	82(29)	139(29)	162(30)	194(30)	223(29)	239(29)	263(20)
37	I	31(20)	59(19)	90(20)	—	—	—	—	—	—
	II	—	—	—	116(20)	158(20)	196(20)	226(20)	239(20)	238(19)
	Mean	..	..	..	..	..	..	..	..	..

---

* For each marking date the number of leaf markers recovered on any sampling date are shown in the final
decomposition or eaten by slugs.

MARKING DATES AT EACH DAY OF HARVEST. THE NUMBER OF PETIOLES IN EACH CLASS IS
PARENTHESIS*

Leaf Age (days)								No. of Leaf Markers Harvested on Each Day*
20	22	24	26	28	30	32	34	
—	—	—	—	—	—	—	—	45
106(27)	125(21)	125(6)	—	—	—	—	—	
96(14)	106(15)	116(17)	131(14)	—	—	—	—	
—	81(4)	108(9)	101(7)	132(6)	—	—	—	
78(1)	—	108(4)	106(10)	105(17)	114(9)	—	—	
102(42)	113(40)	115(36)	116(31)	112(23)	114(9)	—	—	
—	—	—	—	—	—	—	—	45
131(44)	139(40)	155(30)	186(21)	—	—	—	—	
—	160(2)	129(9)	151(12)	147(19)	162(10)	—	—	
—	—	129(4)	129(9)	153(8)	148(4)	—	—	
—	—	—	—	—	122(6)	—	—	
131(44)	139(42)	147(43)	164(42)	149(27)	147(20)	—	—	
—	—	—	—	—	—	—	—	45 except for last two samplings when 30 were harvested
197(43)	225(35)	219(36)	233(37)	229(12)	274(3)	—	—	
—	183(8)	214(6)	—	218(15)	241(17)	291(14)	—	
—	—	—	—	196(8)	237(4)	232(2)	—	
—	—	—	—	—	—	216(4)	—	
197(43)	217(43)	218(42)	233(37)	218(35)	245(24)	270(20)	—	
—	—	—	—	—	—	—	—	45 for first 11 samplings. 30 for next two samplings and 20 for remainder
252(45)	242(44)	267(29)	273(27)	286(12)	285(8)	294(5)	—	
—	—	—	216(2)	—	283(4)	268(11)	277(8)	
—	—	—	—	254(3)	260(5)	—	276(6)	
—			—		205(2)	—	—	
252(45)	242(44)	267(29)	269(29)	279(15)	270(19)	276(16)	277(14)	
—	—	—	—	—	—	—	—	20
293(20)	319(19)	309(19)	325(18)	314(11)	—	—	—	
—	—	—	—	277(5)	292(12)	309(9)	319(3)	
—	—	—	—	—	223(4)	286(5)	298(4)	
—	—	—	—	—	—	—	262(5)	
293(20)	319(19)	309(19)	325(18)	302(16)	275(16)	299(14)	288(12)	
—	—	—	—	—	—	—	—	30 for first 7 samplings, and 20 for remainder.
289(19)	281(20)	307(20)	294(16)	281(18)	267(8)	—	—	
—	—		—		268(8)	315(7)	—	
—	—	—	—	—	306(3)	—	—	
—	—	—	—	—	—	—	—	
289(19)	281(20)	307(20)	294(16)	281(18)	274(19)	315(7)	—	
—	—	—	—	—		—		20
274(20)	261(19)	275(18)	258(18)	265(15)	—	—	—	
,,	,,	,,	,,	,,		—		

column. The difference between this and the total number of leaves harvested were missing through complete

Table 2 shows the amount of nitrogen contained in Class V leaves at different stages of regrowth, expressed as lb per acre.

TABLE 2. TOTAL NITROGEN CONTENT OF DEAD LEAVES

Day of Sampling	Dry Wt of Dead Leaf (g/ft^2)	% Nitrogen	Amount of Nitrogen (lb/acre)
25	0.080	2.58	0.19
35	0.692	2.25	1.54
40	0.793	2.54	1.92
45	0.529	2.77	1.44
55	0.793	2.88	2.21

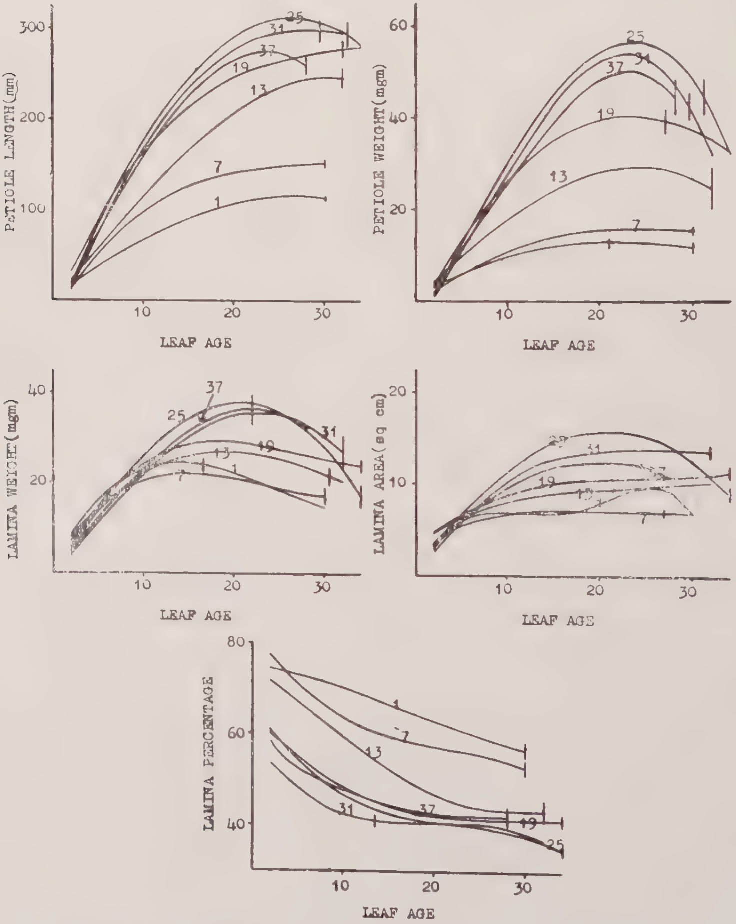


Fig. 2.—Relationships between leaf age and leaf dimensions for each marking day. The average standard errors of estimate are also shown.

These figures were obtained from determination of the total amount of nitrogen in the dried samples of leaves used in the L.A.I. measurements shown in Fig. 1.

Discussion

The total L.A.I. of white clover swards reached a ceiling value of approximately 5.5 twenty days after defoliation (Fig. 1). Thereafter until the end of the experiment, only small changes of less than 1 unit occurred. These results are in agreement with those previously obtained for the growth of this species (Brougham 1958).

This pattern of sward development was determined by the percentage of the total amount of leaf at various stages of development or senescence.

The amount of new leaf (Class I) developing under the sward canopy reached a maximum value of 0.5 L.A.I. units when the ceiling L.A.I. was reached. Little change from this value then occurred for the duration of the experiment.

Similarly, the L.A.I. of Class II leaves (fully opened, actively photosynthesising leaves) reached a ceiling of 3–3.5, twenty days after defoliation, and as with Class I leaves, only slight changes from this value were obtained until the end of the experiment. It is of interest to note that in the previous experiment (Brougham 1958), carried out in the same season of the year (late spring and early summer), the L.A.I. of white-clover stands at which 95% of the incident light energy was intercepted was 3.5.

Senescence of leaves began when the total L.A.I. reached a value of approximately 4. From this it can be deduced that acceleration of senescence was associated with the development of a leaf canopy of sufficient density to intercept almost all the light. Leaf senescence can therefore be attributed to intense shading by the dense canopy of active leaves. Approximately twenty days after defoliation, complete yellowing of some of the first-formed leaves occurred, and by the thirtieth day the amount of leaf in various stages of senescence amounted to one L.A.I. unit. By the end of the experiment this value had increased to 1.5 units. Thirty-five days after defoliation, the amount of dead leaf (0.5 units) was equal to the amount of new leaf being formed, and this relationship between new and dead leaf remained approximately constant for the remaining twenty days of measurement.

The amount of axillary leaf present under the canopy reached a maximum value of approximately 1 L.A.I. unit twenty days from defoliation, and thereafter fell progressively to reach an approximately constant value of 0.5 units thirty days from defoliation. These leaves were much smaller in all dimensions than fully developed leaves initiated from terminal buds, and from previous work (Brougham 1958) the intensity of light received by them would be less than 500 f.c. These results suggest that immediately following defoliation, meristematic activity increased, and was followed by the production of a large bulk of both terminal and axillary bud leaves. However, by the time a ceiling L.A.I. was reached, meristematic activity decreased, and thereafter until the end of the experiment it remained relatively constant. Such increased meristematic activity following defoliation could be associated with increased nutrition of apical growing points (Allsopp 1953), and increased light (Mitchell 1956) following the removal of the existing canopy.

These results, together with those obtained from the previous experiment, show that the ceiling L.A.I. of white-clover stands is determined by the amount of available light energy which influences the amount of active leaf present, together with the rates of initiation of new leaves and death of old leaves. In this experiment the amount of light energy available for growth was sufficient to maintain a canopy of actively photosynthesising leaves of approximately 3–3.5 L.A.I. units. Some small changes in the ceiling L.A.I. of less than one unit were obtained but these are attributed to the influence of prevailing weather conditions on leaf decomposition. Under dry conditions decomposition was delayed, whereas moist conditions led to rapid decomposition of dead leaves.

The results also show that approximately 20% of the total amount of leaf present in the sward is in various stages of senescence. Much of this leaf would contribute little to the build-up of photosynthate as respiratory losses would far exceed their contribution to photosynthesis. This percentage of leaves is similar to that postulated by Davidson and Donald (1958) for subterranean-clover swards. At a ceiling L.A.I. value of 8.5, they considered that about one-third of the leaves present were parasitic.

The pattern of petiole and lamina development of individual leaves initiated at different times throughout the period of the experiment markedly influenced the pattern of sward development outlined above.

The results presented in Table 1 show that the life cycle of individual leaves ranged from 28–34 days regardless of the time of initiation after defoliation. However, observations showed that leaves were marked at a leaf age of 6–8 days, so that the complete life-cycle of leaves from the time leaf buds were visible until death occurred was approximately 40 days. During the life cycle, 10–15 days elapsed before the leaflets of the leaves were fully opened, and senescence began 15–20 days later. With first-formed leaves, senescence was extended over a 14-day period, but, for leaves marked at successive dates thereafter, there was a progressive decrease in the time interval over which senescence occurred.

Following defoliation, the laminae of leaves originating from the first crop of terminal bud leaves reached maximum weight and area at a leaf age of 10–12 days after marking, although the petioles showed increases in both length and weight up to a leaf age of 20–26 days after marking. The laminae of the leaves marked on the next four dates reached maximum dimensions at progressively later leaf ages, whereas the laminae of leaves marked after the ceiling L.A.I. was reached attained maximum dimensions of approximately equal magnitudes 20 days from marking. This trend was not evident for petiole dimensions, for, regardless of marking date, maximum weight was reached at a leaf age of 20–26 days, and maximum length was obtained towards the end of the life cycle.

The maximum weight and area of laminae from leaves marked after the ceiling L.A.I. was reached were twice the size of first-formed leaves, and petiole dimensions were 2.5 and 4 times the length and weight respectively. The dimensions of petioles and laminae of leaves marked on intermediate dates increased progressively to these maximum values.

For most marking dates, lamina dimensions decreased after maximum values were reached. Although a similar trend was obtained for petiole weight, petiole length remained approximately constant after maximum dimensions were attained, for all except the last three marking dates.

These decreases ~~in~~ leaf dimensions are attributed to the remobilisation of chemical constituents to the stolons and growing points of the plants, together with weight losses due to leaching of soluble compounds from leaves and petioles, and slug, worm, and insect damage. For example, the area and weight curves of laminae developing from leaves marked on the second, third and fourth dates show relatively constant areas but decreasing weights over the last half of the life cycle. It can be deduced from this that leaf density decreased over this latter period because of remobilisation or leaching of plant materials to other parts of the plant or to the ground. However, the decreases recorded in lamina area during the last part of the life cycle for a number of the marking dates can be attributed only to slug, worm, and insect damage.

The percentage of the total weight of first-formed leaves contributed by laminae was initially 75, but this decreased to approximately 55 at the end of the life cycle. Leaves marked on the next three dates had progressively less lamina, on a percentage basis, throughout their life cycle than first-formed leaves, whereas only small differences in the lamina percentage were recorded throughout the life cycle of leaves marked after the ceiling L.A.I. was reached.

It is considered that the above pattern of leaf growth was determined largely by the light received by laminae developing under the existing canopy. Following defoliation, the first crop of new leaves form a canopy which intercepts a considerable amount of light. Leaves developing later are therefore subjected to a low light intensity and petiole elongation continues until the laminae of these leaves have penetrated the existing canopy and reach full light. This process is repeated with the result that the petioles of successive crops of leaves show increases in maximum length. At a certain stage of growth (in this experiment approximately 40 days from defoliation) a physical limit to elongation must operate and the petioles of subsequent crops of leaves do not exceed this length. From observations made in mixed swards of grass and clover, a greater petiole length is attained by white clover at comparable stages of growth, because of the support obtained from the more erect leaf blades and stalks of the associated grasses. In this respect N.Z. certified white clover has distinct advantages over other strains with more prostrate growth habits (e.g. Kentish white clover). Its erect growth habit enables it to compete more favourably with associated grasses under relatively long spelling between grazings (Sears 1951).

It is probable in view of the results obtained by Went (1941), Watson (1942), Morton and Watson (1948), Mitchell and Soper (1958), and Bensink (1958), that cell division and elongation occurs at a relatively fast rate during the period that petioles and laminae are shaded. As the petioles increase in length, and the laminae receive increasingly higher light levels, these processes slow down. By the time the laminae are in full light, petiole elongation slows down to negligible rates and photosynthesis then becomes the main activity (Singh and Lal 1935).

Similarly, the opening of laminae of bud leaves is associated with the light received, although the results presented in Table 1 show that opening occurred before the leaves had penetrated the existing canopy. It is probable that once the laminae are placed in an environment where the light intensity is favourable for photosynthesis, then leaf expansion slows down (Hicks 1934; Ashby and Oxley 1935). The results presented in Fig. 2 suggest that the rate of lamina expansion was similar for all leaves regardless of marking date, and that increases in weight and area were determined by the period of time during which expansion occurred. Both the lamina weight and area curves (Fig. 2) show that maximum dimensions were reached at progressively later leaf ages, so that if the rates of lamina expansion were similar, progressive increases in maximum lamina dimensions would be obtained for successive crops of leaves.

In an earlier part of this discussion it was suggested that meristematic activity increased immediately following defoliation, but was followed by a gradual decline in the rate of initiation of bud leaves to reach a constant rate after the ceiling L.A.I. was reached. The results presented above give additional support to this view. Fig. 1 shows that after a ceiling L.A.I. was reached, 20 days from defoliation, the amount of fully opened leaf present was maintained at 3.25 L.A.I. units, i.e. approximately 3.25 sq. ft per sq. ft of pasture. On the other hand, Table 1 shows that, regardless of marking date, leaves were in this state for approximately 20 days of their life cycle. It can be deduced from this that 150 cm^2 of leaf passed from Class I to Class II per day, for each sq. ft of pasture, and that a similar amount of leaf began to senesce daily. From Fig. 2 it can be calculated that the mean area of Class II leaves, 20 days from defoliation, was approximately 7 cm^2, whereas the mean area of Class II leaves, 40 days from defoliation was approximately twice this value. This shows that twice as many leaves were present in the sward 20 days from defoliation compared with 20 days later. It also suggests that the rate of bud initiation immediately following defoliation was twice that of the equilibrium rate of ten leaf primordia per square foot per day.

From similar calculations using the data presented in Fig. 2 and Table 2, an estimate can be made of the amount of nitrogen transmitted from the leaves of white clover stands to the ground, through leaf decomposition. The rate of turnover of the total amount of leaf present in the sward after the ceiling L.A.I. was reached was 1 L.A.I. unit every five days (L.A.I. of 5.5; life cycle approximately thirty days). Thus the rate of decomposition of dead leaf was also 1 unit every five days. From Table 2, the nitrogen content of dead leaf was approximately 2.5 to 2.75, so that the amount of nitrogen present in this amount of dead leaf would be equivalent to 3–4 lb per acre. This nitrogen would be transmitted to the ground surface and would be available either for subsequent growth or lost in drainage water or to the air. In addition, an approximately equal amount was unaccounted for during the process of senescence (the nitrogen content of Class II leaves was 5–5.5%) and it can be assumed that it was either remobilised to other parts of plant during senescence or leached from the leaf to the ground surface or lost to the air. For this reason, the estimate made is a conservative one, although it does emphasise the significance of leaf-fall on the turnover of symbiotically fixed nitrogen. It is conceivable that, in high-producing mixed swards, a considerable portion of the recorded figure of 500–600 lb of nitrogen per acre fixed annually by the clover component (Sears 1953) could be made available to the associated grasses in this manner. However, the quantity returned through this medium would be dependent on a number of factors, particularly the grazing management system. For this reason, additional experimental work is required before its importance in nitrogen turnover can be fully determined.

The above results also have important practical implications, for they show that at a relatively early stage in recovery of white clover from defoliation (20–30 days), a considerable amount of dry matter is lost through death of plant tissue. These losses, particularly under grazing conditions where white-clover-dominant pastures are spelled for long periods before grazing, result in decreased seasonal and annual yields compared with more frequent grazings (Jones 1933; Sprague and Garber 1950). It is also probable that similar results would be obtained for the growth of species such as subterranean clover (Davidson and Donald 1958), and timothy (*Phleum pratense*), where the development of a dense top canopy of leaf at most stages of growth, because of leaf orientation or flagging, would hasten the death of older leaves through shading. For pasture species with narrow leaves and a relatively erect growth form (e.g. *Lolium* spp.), the available light penetrates to the lower layers of the pasture and at most stages of growth a considerable portion of all leaf blades would receive light at a relatively high intensity (Brougham 1958; Mitchell and Calder 1958). Senescence of leaves through intense shading would therefore be reduced, and lengthened spelling of the pasture between grazings would not result in the same loss of production.

ACKNOWLEDGEMENTS

The writer wishes to express appreciation of the assistance obtained from Mr. A. C. Glenday, Biometrician, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, Wellington, in the mathematical treatment of the results. The technical assistance of

Mr. K. Craven, Plant Chemistry Laboratory, Department of Scientific and Industrial Research, Palmerston North, for the nitrogen analyses is also much appreciated.

REFERENCES

ALLSOPP, A. 1953: Experimental and Analytical Studies of Pteridophytes. XIX. Investigations on *Marsela*. 3. The Effects of Various Sugars on Development and Morphology. *Ann. Bot. Lond. n.s. 17:* 447-63.

ASHBY, E.; OXLEY, T. A. 1935: The Interaction of Factors in the Growth of Leaves. VI. An Analysis of the Influence of Light Intensity and Temperature on the Assimilation Rate and the Rate of Frond Multiplication. *Ann. Bot. Lond. 49:* 309-36.

BENSINK, J. 1958: Morphogenetic Effects of Light Intensity and Night Temperature on the Growth of Lettuce (*Lactuca sativa* L.), with Special Reference to the Process of Heading. (Communicated by Prof. S. J. Wellensiek). *Proc. Ned. Akad. Wet. C61:* 89-100.

BROUGHAM, R. W. 1956: Effect of Intensity of Defoliation on Regrowth of Pasture. *Aust. J. agric. Res. 7:* 377-87.

———1958: Interception of Light by the Foliage of Pure and Mixed Stands of Pasture Plants. *Ibid. 9:* 39-52.

DAVIDSON, J. L.; DONALD, C. M. 1958: The Growth of Swards of Subterranean Clover with Particular Reference to Leaf Area. *Ibid. 9:* 53-72.

HICKS, P. A. 1934: Interaction of Factors in the Growth of *Lemna*. V. Some Preliminary Observations upon the Interaction of Temperature and Light on the Growth of *Lemna*. *Ann. Bot. Lond. 48:* 515-25.

JONES, L. I. 1933: The Effect of Varying the Period of Rest in Rotational Grazing. *Welsh J. Agric. 9:* 159-70.

MITCHELL, K. J. 1956: Growth of Pasture Species. III. White Clover (*Trifolium repens*), Subterranean Clover (*T. subterraneum*) and Lotus Major (*L. uliginosus*). *N.Z. J. Sci. Tech. A37:* 395-413.

MITCHELL, K. J.; CALDER, D. M. 1958: The Light Regime within Pastures. *N.Z. J. agric. Res. 1:* 61-8.

MITCHELL, K. J.; SOPER, K. 1958: Effects of Differences in Light Intensity and Temperature on the Anatomy of Leaves of *Lolium perenne* and *Paspalum dilatatum*. *Ibid. 1:* 1-16.

MORTON, A. G.; WATSON, D. J. 1948: A Physiological Study of Leaf Growth. *Ann. Bot. Lond. n.s. 12:* 281-310.

SEARS, P. D. 1951: The Technique of Pasture Measurement. *N.Z. J. Sci. Tech. A33:* 1-29.

———1953: Pasture Growth and Soil Fertility. I. The Influence of Red and White Clovers, Superphosphate, Lime, and Sheep Grazing, on Pasture Yields and Botanical Composition. *Ibid. A35 (Suppl. 1):* 1-29.

SINGH, B. N.; LAL, K. N. 1935: Investigations of the Effect of Age on Assimilation of Leaves. *Ann. Bot. Lond. 39*: 291-307.

SPRAGUE, V. G.; GARBER, R. J. 1950: Effect of Time and Height of Cutting and Nitrogen Fertilisation on the Persistence of the Legume and Production of Orchard Grass—Ladino and Bromegrass—Ladino Associations. *Agron. J. 42:* 586-93.

WATSON, D. J. 1947: Comparative Physiological Studies on the Growth of Field Crops. I. Variations in Net Assimilation Rate and Leaf Area between Species and Varieties, and Within and Between Years. *Ann. Bot. Lond. n.s. 11:* 41-89.

———1956: Leaf Growth in Relation to Crop Yield. Symposium on the Growth of Leaves. *Proc. 3rd Easter School agric. Sci. Univ. Nottingham*: 178-91.

WATSON, R. W. 1942: The Effect of Cuticular Hardening on the Form of Epidermal Cells. *New Phytol. 41:* 223-29.

WENT, F. W. 1941: Effects of Light on Stem and Leaf Growth. *Amer. J. Bot. 28:* 83-95.

THE INSECT COMPLEX OF SOWN PASTURES IN THE NORTH ISLAND

I. THE GENERAL PICTURE REVEALED BY SUMMER SWEEP-SAMPLING

By R. A. CUMBER, Entomology Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 20 June 1958*)

Summary

Techniques developed for the rapid sweep-sampling of pastures are described.

One hundred and thirteen sampling stations based on the various pasture types which occur in the North Island were investigated during the summer of 1957.

Roadsides adjacent to sampling areas have been studied to determine their possibilities as reservoirs of insects.

A general analysis of the insect complex encountered has been made. It is shown that the order of 20 species and 80 specimens per 8 square yards is a common level of occurrence in paddocks and roadsides. Maxima in paddocks of 44 species (excluding Collembola) and 1,300 specimens (excluding Collembola and Thysanoptera) were taken. A population of 2,700 was recorded in the Collembola for one paddock sample.

Collembola, Thysanoptera, and Diptera are shown to be present in greatest numbers. Considerable numbers of Hemiptera and Hymenoptera are also present, however, the remaining orders being represented by relatively few individuals. The greatest number of species taken (excluding Collembola) was in the Hymenoptera being followed by the Diptera, Hemiptera, Coleoptera, Lepidoptera, Thysanoptera, Psocoptera, and Neuroptera in that order. Many species are shown to be of widespread occurrence. The majority of the species taken are represented by relatively few individuals. The importance of the species, however, is not necessarily related to its abundance.

INTRODUCTION

Knowledge of the factors that control pasture productivity must include an understanding of the insect complex. It is to this end that the present work has been undertaken.

The literature on pasture insects in New Zealand contains some 140 references, but most are fragmentary. About 20 species of insects are involved, the more important ones being the grass grub (*Costelytra zealandica* (White)), subterranean grass caterpillars (*Oxycanus* spp.), the common cricket (*Acheta commodus* (Walk.)), and species of army worms.

Attention has been focussed on insect damage only where the outbreaks have resulted in an almost complete destruction of turf and a baring of the soil. Such outbreaks are often sporadic. The complex of pasture plant species with their varying production peaks tends to

N.Z. J. agric. Res. 1: 719–749

mask any damage to individual species. Under these circumstances, less easily detected yet widely distributed insect species could well be more important in overall production than those concerned in the spectacular sporadic insect outbreaks. It seems highly probable that the accepted pasture-plant complexes in the various areas have come to be accepted partly because of their compatibility with the insect complex.

The survey was carried out primarily to determine the identity of the insect species present and secondarily to obtain information on their distribution and abundance. More than 270 species are involved. Some are new to science, others are unrecorded introductions from overseas. The present account, however, deals with the insect complex generally.

TECHNIQUES

A rapid and simple technique for sampling and handling materials was developed in order that one person with a field assistant could obtain a reasonable coverage of the North Island within a limited span of time. Only a single sampling was possible in each area. Sampling commenced in January in the earlier, warmer far northern districts and progressed southwards until completion during the first week of April. The summer of 1957 was singularly fine and suited to such field work.

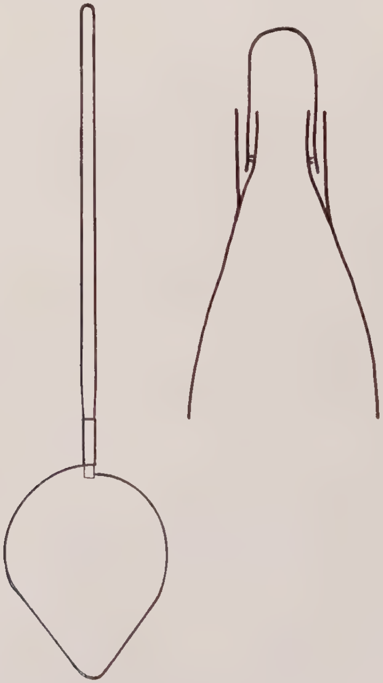


Fig. 1.—The frame of the sweep net (right) is flattened towards its apex. The cross section of the sweep bag (left) and detachable end sock illustrates the double flap and position of dome fasteners.

Collection of Samples

A net with flattened sides running to an apical point was designed. This enabled a swathe of a definite width to be evenly swept, the net being reversed for successive swathes in progressing forward. Samples each comprised 12 sweeps and covered an estimated 8 square yards.

The problem of transferring the catch to the killing bottle without loss was overcome by the use of detachable end-socks. The sweep bag which resembles a rapidly narrowing wind-sock was equipped with a double flap at its distal end. Small muslin socks with dome fasteners and attached to the bag between the flags, proved highly satisfactory (Fig. 1).

Following sweep-sampling, a flick into the wind assured that all material reached the end of the sock. This was rapidly closed off with the hand, detached at the domes, and sealed tightly with a rubber band. The whole sock was then transferred to the killing jar following the application of a few drops of ethyl acetate to the filter paper in the

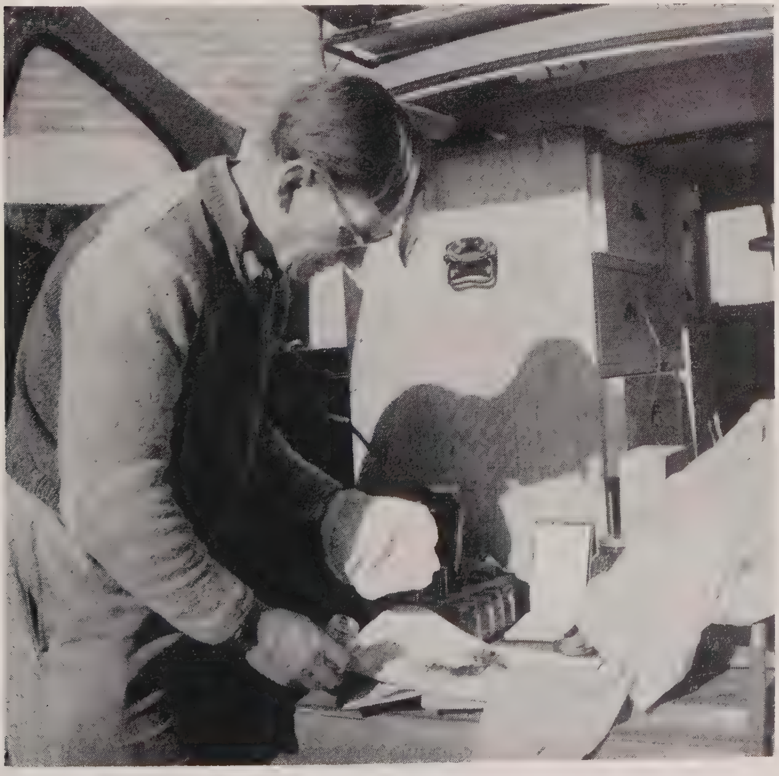


Fig. 2.—After removal of socks from larger killing bottles, the samples are transferred to small holding jars with the aid of a sheet of paper. Coarse foreign material is discarded during the process, which has to be carried out in the absence of wind.

—*Photograph D. Todd*

bottom of the jar. The killed samples were transferred to 2-oz. field holding jars with screw caps, a piece of absorbent paper being added to each (Fig. 2).

Duplicate samples were preserved in 80% alcohol in case of loss amongst the dry material due to mould damage. Fortunately no such losses occurred.

Handling of Collected Samples

It was found desirable to return with material to headquarters at intervals not exceeding two weeks. On return, the samples held in the screw-cap jars were transferred to open petri dishes and held for several days on filter papers for drying out. Following this, the coarse foreign matter such as seed heads, blades of grass and dung fragments were removed. The remainder consisted of insects, fine leaf fragments, seeds, anthers, and mites. Those insects which could be removed macroscopically were separated and kept in covered petri dishes, the fine remaining fraction being transferred to 4-in. by 1-in. corked glass tubes. This fine fraction contained the Collembola, Thysanoptera, mites, and some of the smaller Hymenoptera, and had to be studied under the microscope. The addition of flake naphthalene to all samples ensured their preservation. Species separations and counts were carried out during the winter months.

Coverage Plan

Detailed Survey Stations. Madden (1940) classified and mapped the grassland areas of the North Island. Since the preparation and publication of his work, the boundaries of his pasture types have altered considerably, and in many cases there has been a modification of the composition of the pastures. Large new areas have been brought into production. Nevertheless, Madden's work provided a basis on which the coverage could be planned and it has proved indispensable. His first nine pasture types were selected for study and 56 stations based on the more extensive areas of these types were investigated.

The pasture types and location of these stations were as follows:

"*Type 1.* Ryegrass, white clover dominant."
Hawke's Bay: Napier-Clive.
Gisborne: Gisborne.

"*Type 2.* Ryegrass, white clover, cocksfoot, *Paspalum* as variable dominants with subterranean clover, suckling clover, and *Lotus*".
North Auckland: Helensville.
South Auckland: Tuakau, Morrinsville.

"*Type 3.* Ryegrass, white clover, cocksfoot, with some to much Yorkshire fog, crested dogstail, *Poa pratensis* and suckling clover."
South Auckland: Rotorua.
Taranaki: Manaia.
Wellington: Feilding.
Gisborne: Gisborne.
Hawke's Bay: Dannevirke.

"*Type 4.* Cocksfoot and/or crested dogstail dominant with much Yorkshire fog and white clover: some ryegrass, *Poa pratensis,* suckling clover, and sweet vernal."

South Auckland: Pukekohe, Te Kuiti.
Taranaki: Inglewood.
Wellington: Taihape, Marton, Masterton.

"*Type 5.* *Paspalum* dominant with much ryegrass, cocksfoot, white clover, and possibly subterranean clover and *Lotus.*"
North Auckland: Kaitaia (2 stations), Kaikohe (2 stations), Whangarei, Dargaville (2 stations).
South Auckland: Thames, Whakatane, Opotiki.

"*Type 6.* *Paspalum* invading browntop and/or *Danthonia,* suckling clover, and *Lotus,* with some to much cocksfoot."
North Auckland: Victoria Valley (2 stations), Kerikeri (2 stations), Wellsford.
South Auckland: Tauranga.

"*Type 7.* *Danthonia* dominant, some hairgrass, ratstail, rice-grass, suckling clover, and clustered clover."
North Auckland: Kaeo (2 stations).
South Auckland: Pukemiro.
Wellington: Castlepoint.
Hawke's Bay: Porangahau, Mohaka.
Gisborne: Tolaga Bay.

"*Type 8.* *Danthonia* and/or browntop dominant, improved pastures holding some crested dogstail, cocksfoot, suckling clover, *Lotus major,* subterranean clover, ryegrass, and white clover."
North Auckland: Warkworth, Helensville.
Wellington: Himatangi.
Hawke's Bay: Hastings, Norsewood.

"*Type 9.* Browntop dominant, some sweet vernal, Yorkshire fog, suckling clover, *Lotus,* crested dogstail, and probably *Danthonia.*"
North Auckland: Whananaki, Silverdale.
South Auckland: Paparata, Te Kuiti-Awakino.
Taranaki: Tarata.
Wellington: Taihape, Carterton, Eketahuna (2 stations).
Hawke's Bay: Te Pahue.
Gisborne: Tiniroto, Te Karaka.

The above 56 stations were selected for detailed study. At each station the pasture, and in addition the adjacent roadside where suitable, were swept. Sweeping of pastures was carried out at a distance from boundaries and wherever possible under conditions of medium grazing. It was considered that herbage of this height would provide the best representative insect catch. In many cases the roadside herbage approximated the condition of longer closed pasture. Sometimes, however, especially in the *Danthonia* and browntop areas, there was little difference between roadside and paddock. Dusty roads and the shrubby nature of roadside cover were complicating factors, non-grassed and excessively dusty roadsides not being sampled.

In addition to sweeping, an examination of the soil, turf, and dung was made at each of these detailed survey stations (Fig. 3). Six random spade-square samples about 8 inches in depth were closely examined and insect materials were recorded and retained for determination. In the case of cattle-grazed pastures, 10 dung pats were examined. In the initial stages of the survey, turf samples were collected and taken back to headquarters in emergence cans. These, however, did not yield information of value comparable with the work of handling them, so their collection was discontinued. The results of the soil and dung sampling are to be communicated later.

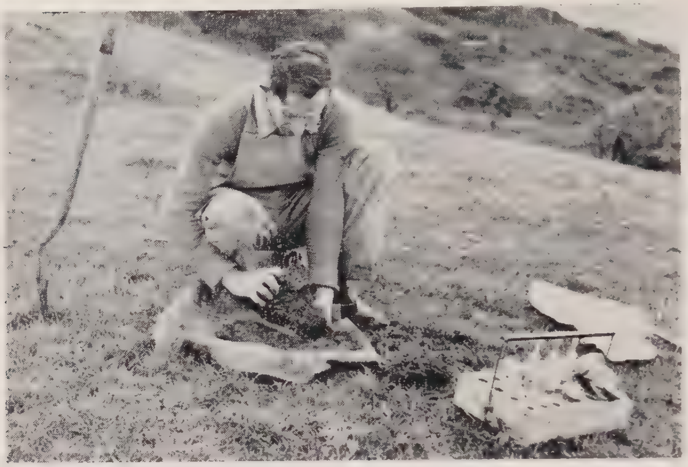


Fig. 3.—Examination of turf and soil at a detailed survey station in *Paspalum* dominant country.

Rapid Survey Stations. The efficiency and speed of sweep sampling was so evident from an early stage that it was decided to supplement the detailed survey stations with a series of rapid survey stations where sweep sampling only was carried out. In all, 57 rapid survey stations in addition to the originally planned 56 detailed survey stations were sampled, giving considerably greater coverage through the study of intermediate areas. Following the detailed station survey in North Auckland in early January, the rapid survey was carried out from Auckland southwards. This produced a North-South survey from Awanui to Paekakariki in the short span of several weeks and in continuously fine weather. This was the closest approach possible to a nullification of the time factor in surveying the widely separated areas.

At each station, whether detailed or rapid survey, details such as location, topography, nature of road (sealed or dusty), pasture composition, type of grazing, time of day, weather, and presence of larger elusive insect species were recorded, and, where the information was available, preceding weather conditions were noted.

Much of this information is included in the Appendix. This should prove valuable in future studies of individual insect species. The location of the sampling stations is shown in Fig. 1, numbers 1–56 being the detailed and 57–113 the rapid survey stations.

The Efficiency of the Sweeping Technique

The efficiency of sweeping varies with the varying habits of the individual species, being least efficient in the case of cryptic insects such as those Collembola that live close to the surface of the soil, and most efficient in species that are not easily disturbed and for the greater part of the day tend to perch on the higher portions of the herbage. Efficiency for the individual species is thus closely related to the length of the herbage when sweep sampling is employed. For this reason,

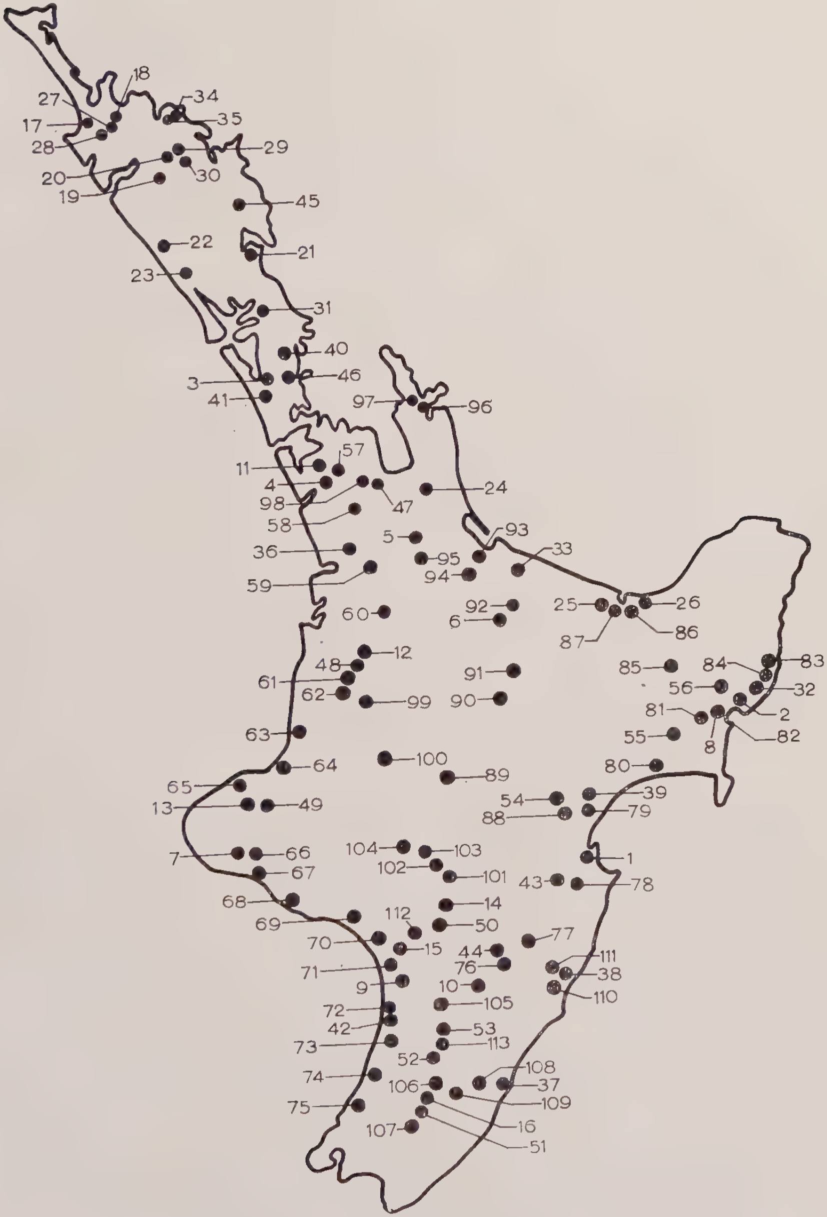

18
27
34
17
35
28
29
20
30
19
45
22
21
23
31
40
3
46
41
97
96
57
11
4
24
98
47
58
93
5
33
36
95
94
59
92
25
26
60
6
87
86
12
48
91
85
84
83
61
56
32
62
99
90
81
2
63
55
8
82
100
64
89
80
65
54
39
13
49
88
79
104
103
7
66
1
102
67
101
43
78
68
14
112
69
50
77
70
15
44
111
76
38
71
9
10
110
105
72
42
53
113
73
52
108
74
106
37
109
75
16
107
51

Fig. 4.—Map to show location of survey stations. Numbers 1-56 indicate the approximate positions of the detailed survey stations, and numbers 57-113 those of the rapid survey stations.

where it has been possible, pastures subject to medium grazing were selected, and sampling was carried out during the warmer daylight hours. As indicated previously, the ungrazed roadsides simulated the longer pastures in many cases.

In comparing the numbers of a species found in paddocks and roadsides, favourability of habitat and efficiency of technique interact, and their relative importance may be determined only by close study.

Two species of Diptera may be used to illustrate typical differences in numbers taken, one favouring the paddock, the other the roadside. Fig. 5 concerns the species *Sarcophaga milleri* J. et T. This species breeds in dung and occurs commonly in grazed pastures. It is an elusive species, however, and is more difficult to net under open, closely grazed conditions. During rest and for mating, or during periods of strong winds, this species congregates among the taller roadside herbage especially where the adjacent paddock cover is closely grazed. This habit is reflected in the frequency distribution of numbers taken in paddocks and adjacent roadsides, the latter producing the greatest number of individuals although the species was present in 88/113 paddock and 87/108 roadside samples.

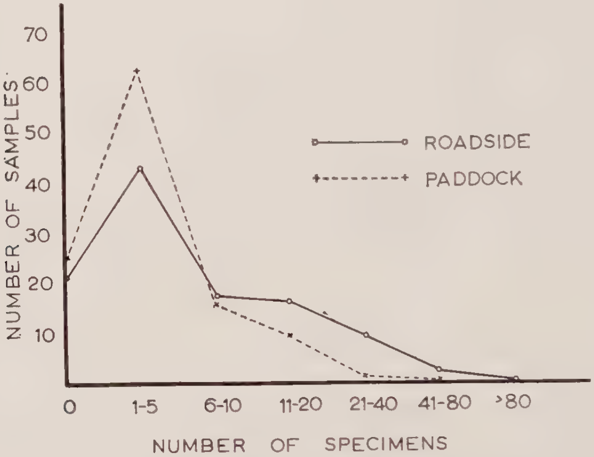


Fig. 5.—Frequency distribution showing sample representation in the large fly *Sarcophaga milleri*. Sweep sampling is more productive in roadsides.

Fig. 6 concerns the smaller species *Hydrellia tritici* Coq. whose habits are not so well known as those of the former example. The frequency distribution shows a markedly greater number taken in the paddocks than in roadsides, this species being present in 91/113 samples in the former as compared with 63/108 in the latter. It is probable that this small species ranges less than the former large species, and that the lower herbage provides sufficient shelter during the more inactive periods.

In order to test the general efficiency of the sweep net on a pasture of medium length, four successive sweepings were made over the one 8-square-yard area at station 9. The total number of insects taken in each sweep were 138, 45, 17 and 12 respectively. Under such conditions it appears probable that two-thirds of the sweepable insects are

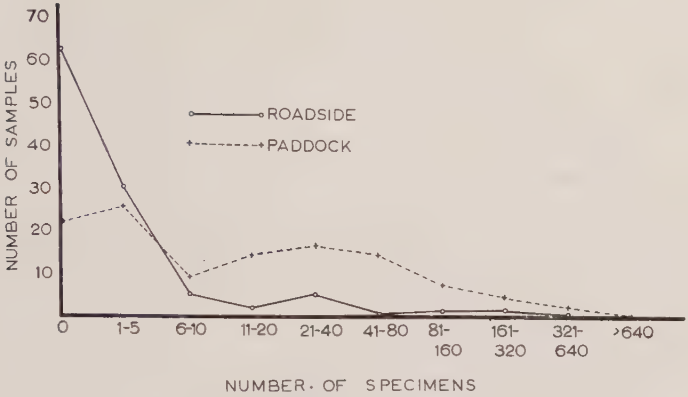


Fig. 6.—Frequency distribution showing sample representation for the small fly *Hydrellia tritici.* Catches are markedly greater in the paddock samples.

taken during the first sweep. The efficiency may be higher, however, on account of the continual movement and quick re-occupation of depleted areas.

Insect populations also vary considerably in different parts of the one paddock as indicated by the following 8 random samples which yielded 141, 124, 62, 323, 128, 88, 109, 91 insects respectively.

ANALYSIS OF INSECT REPRESENTATION

The analyses presented are based on 113 paddock and 108 roadside samples. As already indicated, roadsides adjacent to selected pasture stations were in some areas unsuited to sweep sampling. The information on Collembola and Thysanoptera is incomplete, the limitations in respect of each being indicated.

Numbers of Species and Specimens per Sample

Fig. 7 shows a frequency distribution of the numbers of species and specimens taken in samples from paddocks and roadsides. In the analysis of number of species, the Collembola are excluded, and both Collembola and Thysanoptera are excluded in respect of number of specimens.

In comparing the curves for paddock and roadside, it is seen that they are somewhat similar in shape, but that there is a movement to the right in the paddock data. With the scales adopted, this shift, in the case of the number of specimens represents a considerable increase, and shows that pastures are numerically far richer in total specimens than are the roadsides. The actual numbers of insects taken were 17,400 and 7,400 respectively (nearest 100). The species data do not vary greatly between paddock and roadside, however. Some 275 species were encountered, 225 occurring in the paddock samples and 220 in the roadside samples (nearest 5 species).

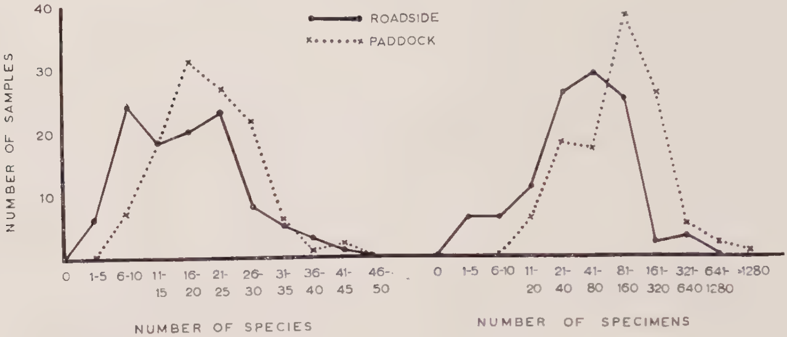


Fig. 7.—Frequency distribution of numbers of species (excluding Collembola) and specimens (excluding Collembola and Thysanoptera) taken in paddock and roadside samples.

The number of species per sample ranged from 8 to 44 in paddocks, and from 1 to 42 in roadsides, and it is seen that the frequency peak is in the vicinity of the 20 species mark. The number of specimens per sample ranged from 17 to 1,304 in paddocks and from 2 to 464 in roadsides. The frequency peak is at the 81–160 specimen mark in paddocks, and 41–80 in roadsides.

Numbers of Specimens Taken in the Orders

Fig. 8 shows the numbers of specimens taken in the different orders for paddocks and roadsides. Collembola and Thysanoptera are excluded.

Differences in the numbers of insects taken from paddock and roadside are apparent in some orders. This is considered later.

It is seen that the Diptera are by far the strongest numerically. In paddocks they comprised 12,000 of a total of 17,400 specimens, and in roadsides 4,200 of a total of 7,400 specimens. Together this constitutes some 65% of the total catch. The Hemiptera follow with 3,800 specimens, and then the Hymenoptera with 2,900 specimens. The other orders are present in relatively small numbers. It seems probable that had the figures for Collembola and Thysanoptera been available, they would have approached those for the Diptera.

Order Specimen Maxima Met in Samples

Fig. 9 shows the greatest number of specimens belonging to the one order encountered in any one of the paddock samples. All orders present are represented here. Counts were made of Collembola and Thysanoptera in the single samples which appeared to have the greatest numbers present. The Collembola were predominantly a single species belonging to the sub-order Symphypleona.

Prominence is given here to the numerical importance of the Collembola and Thysanoptera even though the number of species involved is relatively small. These two orders predominate, together with the Diptera, Hemiptera, and Hymenoptera.

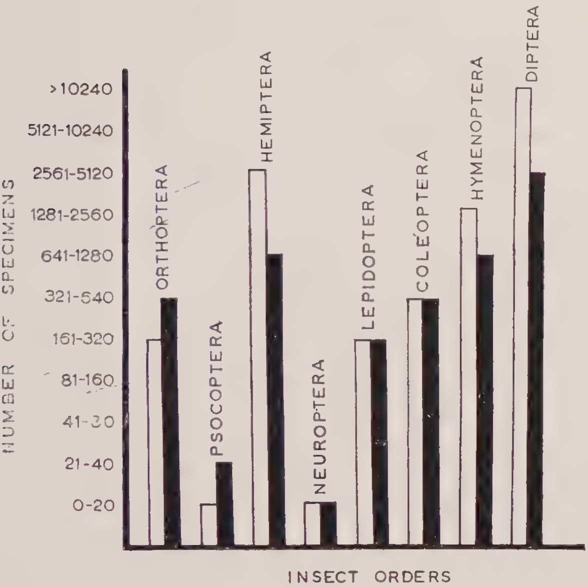


Fig. 8.—Histogram showing number of specimens (all samples) taken in the various orders (excluding Collembola and Thysanoptera) for paddock (open columns) and roadside (black columns).

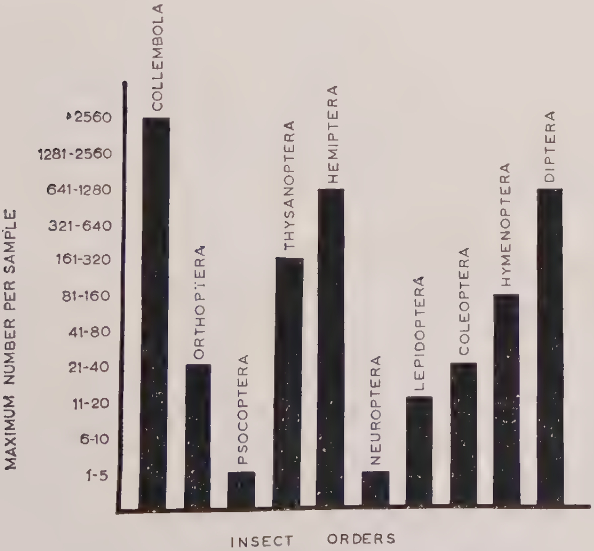


Fig. 9.—Histogram showing the greatest number of specimens of each order found in a single sweep sample.

Species Representation in the Orders

Fig. 10 shows the number of species taken in the various orders with the exception of the Collembola which are estimated at about 20 species. The Hymenoptera present the greatest number of species although in the number of individuals they are exceeded by the Collembola, Diptera, Hemiptera, and Thysanoptera. This species predominance in the Hymenoptera is of considerable interest as the majority are parasitic. This, together with the relatively lower specimen representation, is consistent with the idea of a strong biological balance in the pasture-insect complex. Such orders as the Hemiptera, Neuroptera, Coleoptera, and Diptera are also involved in biological control. These factors strongly suggest that many of the seemingly unimportant low-density species may remain as such only so long as upsets in the control complex are avoided.

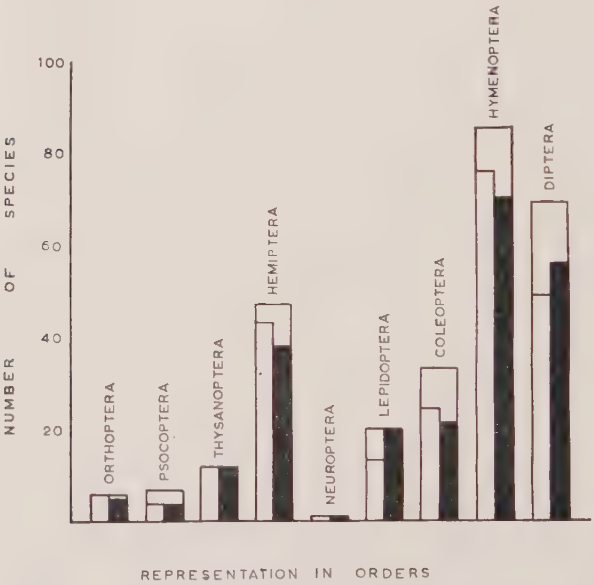


Fig. 10.—Compound histogram showing the number of species in each order (excluding Collembola) taken in paddocks (open columns), roadsides (black columns) and both considered together (combined extended columns).

The numbers of species taken from paddocks, roadsides, and both considered together, are indicated in Fig. 10. The picture of order preferences is a very general one, however, as differences between species within the order may be considerable, as is indicated in an earlier section.

In many cases the pasture-plant species are represented on roadsides, and quite often additional plants not encountered in paddocks are present. On the basis of this, and shelter values, higher species representation might be expected in the roadsides. This has not always been the case however. One contributing factor is the relative efficiency of netting low-living species from short or long herbage.

If one assumes the similarity of "Hedgerows" and roadsides, the results of the present New Zealand survey do not entirely agree with the statement of Osborn (1939) that "The insect population will usually be found much richer here than in the open field."

Small numbers of species of Orthoptera, Psocoptera, and Neuroptera are concerned. The Thysanoptera on account of their smaller size are equally at home in short or tall herbage. Succulence and seeding are important factors. In the Hemiptera, Coleoptera, and Hymenoptera, more species are recorded from paddocks, whereas in the Lepidoptera and Diptera, the roadsides produced the greater number of species. In no order, however, are the differences between paddock and roadside very marked. Doubtless severe grazing conditions would accentuate the advantages of adjacent roadside cover.

Frequency of Occurrence of Individual Species

Fig. 11 indicates the frequency of occurrence of the species in paddocks and roadsides (excluding the Collembola). In paddocks this ranges from 143 species occurring 1–5 times to 1 species more than 95 times. Forty-three species occur between 11 and 35 times in the total of 113 samples. In roadsides, the range is from 130 species occurring 1–5 times to 1 species 86–90 times. Thirty species occur between 11 and 35 times in the total of 108 samples. It is seen that a large number of species are present throughout the North Island.

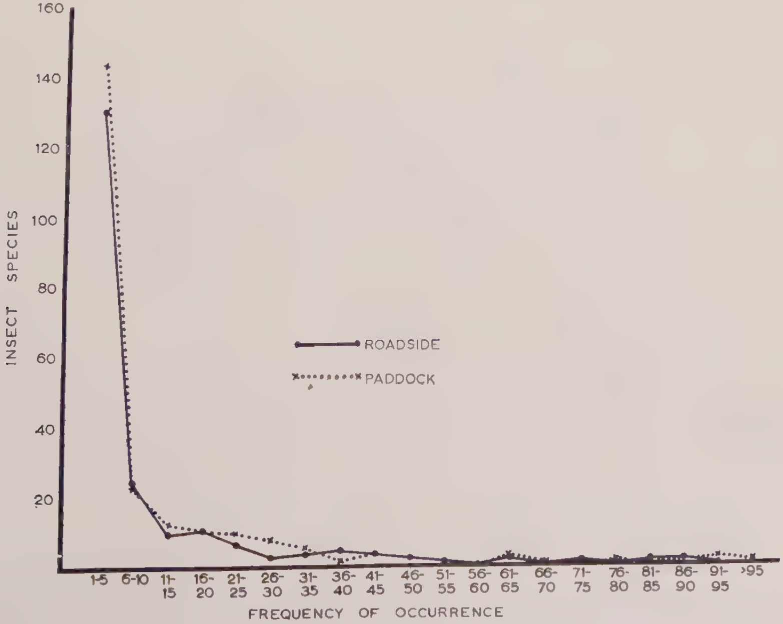


Fig. 11.—Frequency distribution showing sample occurrences of species (excluding Collembola) in paddocks and roadsides.

Total Specimens per Species

Fig. 12 shows the total number of specimens for individual species taken in paddocks and roadsides (excluding Collembola and Thysanoptera). The curves for paddock and roadside are similar. In paddocks, 143 species and in roadsides 141 species are represented by fewer than 11 specimens each. In paddocks 7 species and in roadsides 4 species are represented by more than 321 specimens. This indicates that the majority of the species are represented by a relatively few specimens. However, both paddock and roadside have some 60–70 species which are represented by 11–320 specimens.

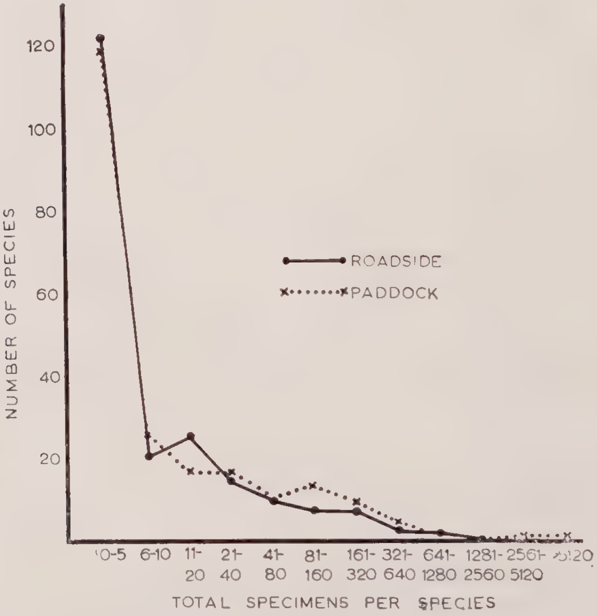


Fig. 12.—Frequency distribution showing number of specimens of species (excluding Collembola and Thysanoptera) in paddocks and roadsides.

Order Representation in the Samples

Frequency distribution graphs are given for each order, with the exception of the Collembola, to show sample representation in species and specimens (except Thysanoptera) for paddocks and roadsides.

Orthoptera. Fig. 13. Five species are concerned, all being taken in paddocks and 4 in roadsides. One of the more widespread and damaging species, the common cricket, was not taken during sweeping. This insect is usually more evident at dusk, although its presence during the day is often indicated by song, or by a few wandering individuals where populations are particularly high. Another elusive species, the migratory locust, was seen on several occasions, but no high populations were observed.

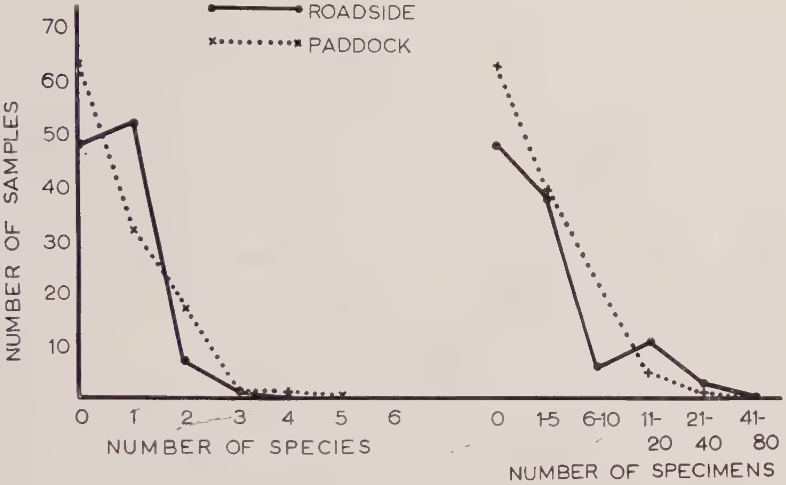


Fig. 13.—Frequency distribution showing sample representation for species and specimens in the Orthoptera.

The curves for paddock and roadside do not differ greatly. Absence of species occurred more times in paddocks than in roadsides, twice as many specimens being taken in roadsides as in paddocks. This picture is strongly influenced by a single, widespread, long-horned

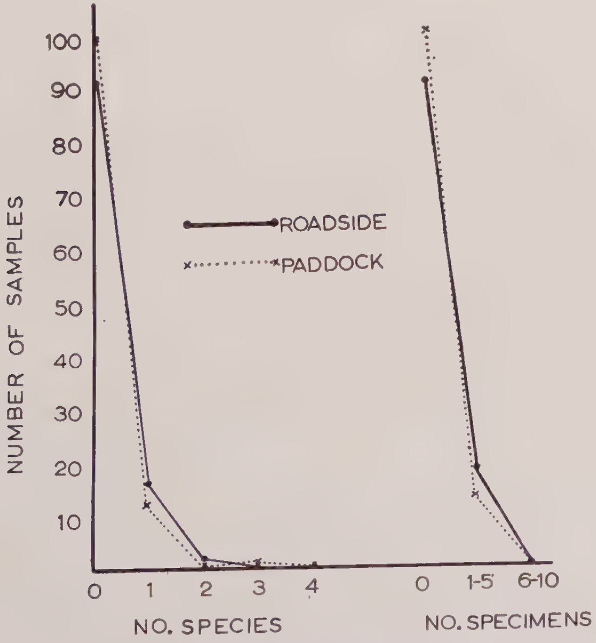


Fig. 14.—Frequency distribution showing sample representation for species and specimens in the Psocoptera.

species which shows a decided preference for the taller and undisturbed herbage. It is probable, however, that the long-horned species (in this instance 4 out of 5) generally prefer the longer herbage.

Psocoptera. Fig. 14. Seven species are concerned, 4 occurring in paddocks and 4 in roadsides. The habits of Psocoptera generally are such that they tend to be found in undisturbed situations, although they are susceptible to dispersal by wind. This latter is well illustrated in the case of *Myopsocus novae-zealandiae* Kolbe, our largest species, which normally is found under bark or in sheltered wood crevices. Dispersal flights resulted in the species being swept from pasture at a distance of several hundred yards from a pine plantation.

The relatively reduced ability of the sweeping technique to reach the more cryptic species in longer herbage, is probably balanced by the dispersal factor and the relatively increased efficiency of the net under conditions of shorter paddock herbage. The difference in number of specimens taken from each site is not marked.

Thysanoptera. Fig. 15. It has not been possible to make sample counts except to ascertain possible maxima. Fig. 15 thus concerns only the number of species per sample.

The frequency distributions in this order illustrate the consistent representation of a relatively large proportion of the small total number of species encountered.

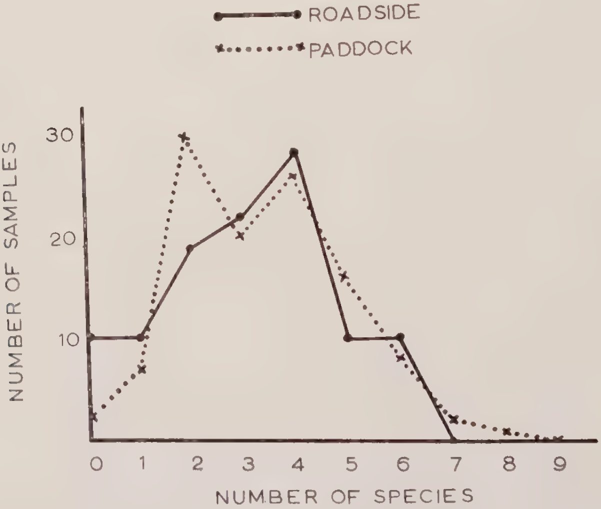


Fig. 15.—Frequency distribution showing sample representation for species in the Thysanoptera.

The curves for paddock and roadside do not differ greatly. Twelve species are concerned, and all were taken in both paddock and roadside. It is seen that only a small number of samples contained no species, this being lower in paddocks than in roadsides.

Hemiptera. Fig. 16. Forty-seven species are concerned, 44 occurring in paddocks and 38 in roadsides. The absence of species is much more frequent in the case of roadside samples. Paddocks show a consistently larger number of samples with 5–9 species present.

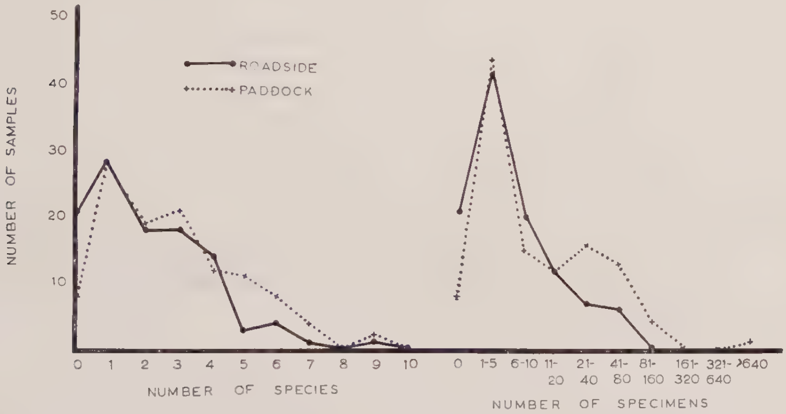


Fig. 16.—Frequency distribution showing sample representation for species and specimens in the Hemiptera.

Paddocks produced almost 3 times as many specimens as did roadsides. This is largely due to the high populations of aphids and jassids met in a relatively small number of paddock samples. This is reflected in the paddock curve between the 21 and greater-than-640 specimen levels. This is a very significant part of the curve. Represented here are species which are of considerable importance not only through the mechanical damage which they cause, but also through their ability to transmit a wide range of virus diseases. It seems probable that production, and the development of high-producing strains in clovers and grasses may be strongly influenced by virus infections. A recognition of this fact and of the viruses and vectors concerned will greatly assist in the problems which arise.

Neuroptera. Only one species is concerned, 12 specimens from paddocks and 17 from roadsides being taken. The species is an important biological control agent.

Lepidoptera. Fig. 17. Twenty-one species are concerned, 13 being taken in paddocks and 20 in roadsides. Roadsides produced almost twice as many specimens as did paddocks.

It is possible that night sweeping might have produced considerably more material. Roadside samples were more consistent in producing representatives of the order.

Coleoptera. Fig. 18. Thirty-two species were taken, 24 in paddocks and 21 in roadsides. The curves do not vary greatly between paddock and roadside. No great population comparable with the case of some other orders was evident in any one sample. The importance of the

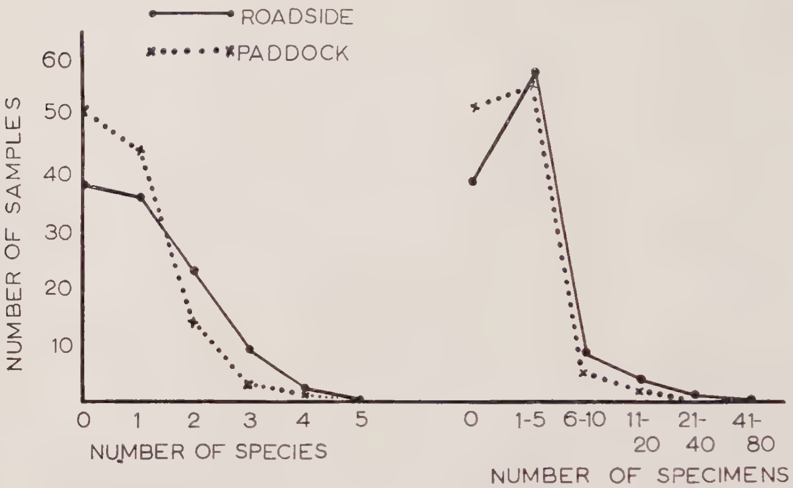


Fig. 17.—Frequency distribution showing sample representation for species and specimens in the Lepidoptera.

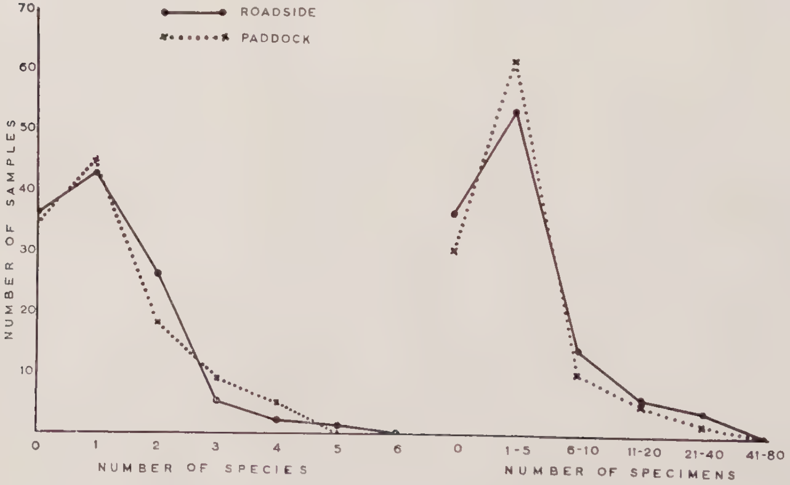


Fig. 18.—Frequency distribution showing sample representation for species and specimens in the Coleoptera.

Coleoptera is perhaps, better illustrated in the results of soil and turf sampling which was carried out simultaneously.

Hymenoptera. Fig. 19. A considerable amount of material is involved here so that more precise comparisons of paddock and roadside are possible. About 84 species are involved, 74 being taken in paddocks and 68 in roadside samples.

The superiority of the paddock samples especially in specimens is quite marked, the number taken being almost double that taken in

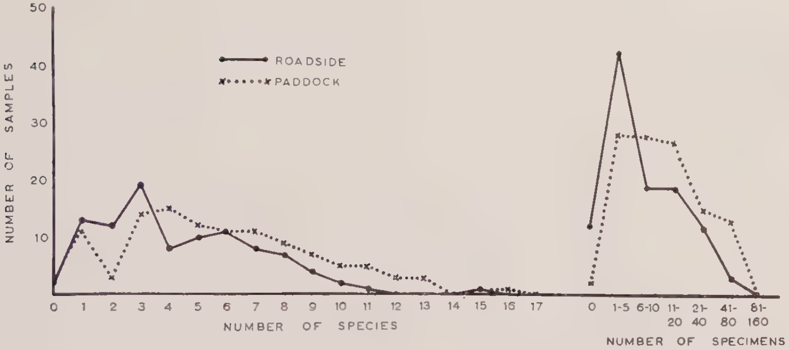


Fig. 19.—Frequency distribution showing sample representation for species and specimens in the Hymenoptera.

roadsides. This could be due in part to the small nature of the majority of the species and the difficulty of netting the more cryptic ones from longer herbage.

An outstanding feature is the low number of samples both from paddock and roadside that contain no representative of the order. A large number of species is concerned, and yet a relatively small number of specimens is concerned. As indicated earlier, these facts are in accordance with a situation where a large number of parasitic species is in balance with the attendant host species.

Diptera. Fig. 20. As in the Hymenoptera, a considerable amount of material is involved. Of a total of some 68 species, 48 were taken from paddocks and 54 from roadsides. However, samples from paddocks are shown to be consistently richer both in species and in specimens. Very few samples did not contain representatives of the order.

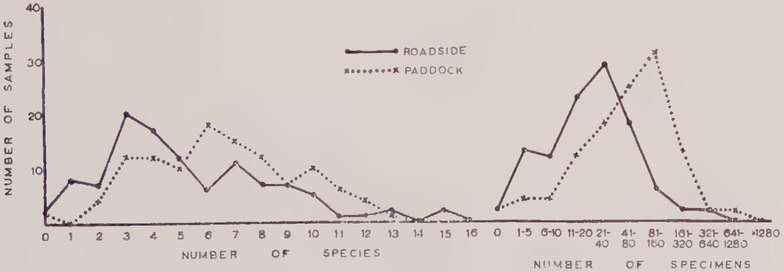


Fig. 20.—Frequency distribution showing sample representation for species and specimens in the Diptera.

The numerical dominance of the Diptera amongst the orders (Collembola and Thysanoptera excluded) is marked.

It is of interest to compare Figs. 19 and 20. The curves for species are somewhat similar in the Hymenoptera and Diptera. The curves for the number of specimens however, show marked differences, that in the Hymenoptera cutting out at the 41–80 mark, whereas the Diptera proceed to the greater-than-1,280 level.

DISCUSSION

It is common knowledge amongst entomologists associated with herbage problems that a complex group of species is usually concerned. This is indicated in such accounts as those of Johnson *et al.* (1957), Osborn (1939) with its full bibliography, Quinton (1956), and Wolcott (1937), but no attempt has hitherto been made to analyse in detail the insect complex as a whole over reasonably extensive areas. The present work attempts this while recognising the limitations to the results set by available facilities. The study and understanding of the complex does not promise immediate answers to specific insect problems, but it would seem to be a basic approach to the subject and one that should be studied.

This approach no doubt has been neglected partly because public opinion demands immediate action on obvious specific problems, and partly because the task in its simplest possible form is a formidable one. Osborn (1939) in his very considerable contribution to the subject of pasture insects indicates the difficulties associated with the study of a single area when he states: "The determination of the relative abundance of different organisms occurring in the meadow, that is, a quantitative study of these organisms, is a matter of much difficulty. It is obvious that determinations in any one place or for any one season cannot be assumed to represent the general condition for different localities at different periods of the year or even for meadows of different history in the same locality. Moreover, the determination for any one locality is a matter of considerable difficulty involving as it does methods by which to secure from an adjoining large area all of the organisms which normally occur there and then to sort and count these in such a manner as to get accurate results. Bearing in mind that these organisms occur at the rate of many millions to the acre, it is at once evident that to take such a census even for a few square feet is a matter of much time and labor."

The difficulties associated with obtaining a picture of the pasture-insect complex over a wide area are immeasurably greater. Although the difficulties impose limitations on the completeness of results, they do not prevent the gathering of a great deal of useful information.

Ideally each station should be sampled at approximately the same time and at intervals of about one month over a period of at least one year. A highly efficient extraction method such as devised by Johnson *et al.* (1957) should be employed. In addition there should be facilities for the rapid and accurate identification of all species taken. Obviously these ideals cannot be attained. Facilities have sufficed for only one sampling at each station. The apparatus used was designed to strike a balance between efficiency, speed in use, and portability. The identification of all material is a long and difficult task. Species new to science are involved, and in some cases specialists from overseas have to be consulted.

It has been assumed that the greatest number of species will be nettable about midsummer so that the survey is best carried out at this time. By commencing in the far north and working southward it is possible to take advantage of the geographical spread of the Island and obtain some nullification of the seasonal factor.

It is probable that the techniques and coverage adopted in the present work have enabled the majority of the important pasture species to be taken. The peak emergence cycles of many insect species occupy relatively small periods, however, and such peaks may have been missed in many cases. Because of this and the fact that many parasitic species often balance with their hosts at relatively low population levels, the presence of a species in low numbers in the present survey cannot be taken to indicate that it does not at one time or another exist in high populations or that the species is necessarily unimportant. In the case of those species that have appeared in considerable numbers in the survey, however, a more definite appraisal is possible. The survey thus does not put each species in perspective, but it does elucidate the complex and indicate many of the species concerned. It shows the presence of certain previously undetected overseas species, and indicates that certain endemic species have reached population levels at which they are suspected of being economically important. This will be considered in later papers which indicate the identity and distribution of individual species.

Conditions at the time of sampling and the composition of the pastures and roadsides at the sampling stations have been given in detail in the Appendix so that in future studies of individual insect species information on possible host plants and seasonal cycles is available.

If the Collembola and Thysanoptera are included, it is estimated that the paddocks have yielded more than 20,000 insects belonging to over 230 species, the sampling area in aggregate being approximately 900 square yards or one fifth of an acre. In a slightly smaller area, roadsides have yielded more than 10,000 insects belonging to approximately the same number of species. This is to be regarded as the average picture in respect of number of specimens. In the Collembola alone, however, one sampling indicated at least 2,700 individuals from 8 square yards which is equivalent to approximately one and a half millions per acre. It seems probable that insect populations exist which are in excess of those encountered.

It should be noted that some of the species of insects encountered will be associated with the various weeds which inevitably punctuate pastures and roadsides to varying degrees.

McAtee (1907), cited by Osborn (1939), studied a meadow in spring and recorded the following numbers of specimens from 4 square feet: Coleoptera 61, Hemiptera 20, Hymenoptera 940, other arthropods 127, cocoons and eggs 20. The Hymenoptera comprise mainly ants. Osborn (1939) refers to the unpublished data of A. E. Miller who recorded the following number of species from the fields at the Ohio State University in 1920-21: Orthoptera 37, Hemiptera 50, Coleoptera 190, Diptera 127, Hymenoptera 117.

Populations of 6½ millions per acre have been recorded for grasshoppers by Ball (1915), and Cowan and Shipman (1947) estimated that crickets destroyed 120 tons of forage in a 640 acre area which is sufficient to carry 44 head of cattle for a period of 9 months. Reports such as these, together with the apparently lower number of insect species in our pastures suggest that problems in New Zealand may be less acute than in other countries.

It is seen that, generally, the insects found in paddocks are present in roadsides. Under conditions of close grazing, heavy winds, or shallow flooding, it seems probable that advantage is taken of the shelter provided by the taller roadside cover, and that such areas may be regarded as insect reservoirs.

In considering the relative desirability of such rough areas, there are two sides to the question. Where land is periodically ploughed and prepared for crops, the beneficial effects of tillage to destroy the insects pests is reduced if recolonisation is made easy through adjacent rough cover. If, however, the undesirable species are controlled in part by a complex of parasites, then the reservoir will act as such for both pest and parasite.

This factor of biological balance in the complex of pasture insects extends further than the control of insects by other insects, for mites, spiders, centipedes, and vertebrates are concerned. It is an insufficiently stressed aspect of the problem, and especially important in the case of endemic potential pest species.

It is thus seen that areas containing interspersed roughage may have certain advantages over larger continuous clean areas in that there is likely to be stability in insect populations from interspersed nuclei of natural control agents.

As knowledge accumulates on the life cycles of the individual species, it will be possible to evaluate more accurately the overall importance of each, either as a direct destroyer of herbage, or as a controller of the associated damaging species.

In economies where both poor and bumper production have their problems, it would seem that a relatively stable and moderate level of production has much to commend it. Returns from the land must be balanced in part by returns to the land. The increasing cost of fertilisers gives greater importance to that natural but relatively slow contribution to fertility resulting from the weathering and decomposition in the soil. These factors, no doubt, were among those which prompted Osborne (1939) to write: "The bearing of the ecologic method of study has been indicated by the writer (Osborn 1917), and with further study of the subject the conviction is emphasised that any adequate control of meadow pests, especially for permanent meadows or pastures, must be based upon a careful examination of the combination or aggregation of organisms in this complex rather than by efforts to find remedies for single species of pests."

ACKNOWLEDGEMENTS

Assistance in field work was given by the staff of the Entomology Division Substation, Palmerston North. Equipment was constructed with the help of the Grasslands Division workshops, Palmerston North, and Mr. H. Drake, Photographer of the same Division, assisted in reproducing all figures.

REFERENCES

BALL, E. D. 1915: Estimating the Number of Grasshoppers. *J. econ. Ent. 8:* 525-27.

COWAN, F. T.; SHIPMAN, H. J. 1947: The Quantity of Food Consumed by Mormon Crickets. *J. econ. Ent. 40:* 825-28.

JOHNSON, C. G.; SOUTHWOOD, T. R. E.; ENTWISTLE, HELEN M. 1957: A New Method of Extracting Arthropods and Molluscs from Grassland and Herbage with a Suction Apparatus. *Bull. ent. Res. 48:* 211-18.

MADDEN, E. A. 1940: The Grasslands of the North Island of New Zealand. *N.Z. Dep. sci. industr. Res. Bull. 79:* 45 pp.

MCATEE, W. L. 1907: Census of Four Square Feet. *Science n.s. 26:* 447-9.

OSBORN, H. 1917: Control of Meadow and Pasture Insects. *Ohio agric. Exp. Sta. Mon. Bull. 20:* 268-73.

——— 1939: "Meadow and Pasture Insects." 288 pp. The Educators' Press, Columbus, Ohio.

QUINTON, R. J. 1956: The Forage Insect Problem. *Conn. agric. Exp. Sta. Circ. 197.* 14 pp.

WOLCOTT, G. N. 1937: An Animal Census of Two Pastures and a Meadow in Northern New York. *Ecol. Monogr. 7:* 1-90.

APPENDIX. DETAILS OF SAMPLING STATIONS

								CLOVERS**											GRASSES**													MISC.
STATION NUMBER A, Paddock; B, Roadside	LOCATION	DATE (1957)	TIME Nearest daylight hour	WEATHER A, Sunny; B, Cloudy; C, Strong Wind	TOPOGRAPHY F, Flat; H, Hilly; R, Rolling; S, Sealed; U, Unsealed rd.	SOIL MOISTURE D, Dry; M, Moist; W, Wet; X, Excessive at times	GRAZING C, Cattle; H, Horses; S, Sheep; L, Close; M, Medium	*Lotus* spp.	Red	Strawberry	Subterranean	Suckling	White	Browntop	Cocksfoot	Crested dogstail	Crowfoot	*Danthonia* spp.	Five-finger	Meadow foxtail	*Paspalum dilatatum*	*Phalaris* spp.	*Poa* spp.	Prairie	Ratstail	Rye	Sweet vernal	Tall fescue	Timothy	Yorkshire fog	B, Blackberry; F, Bracken fern; G, Gorse; R, Rushes; T, Titree
1 A	Napier, Kennedy Road	11.2	2	A	F	D	CM		3	4			1													2					
B	Napier, Kennedy Road	11.2	2	A	S	D							3						1									2			
2 A	Gisborne, Grey's Bush Rd	13.2	9	A	F	DX	CM			5			1		3						4					2				6	
B	Gisborne, Grey's Bush Rd	13.2	9	A	S	DX							2	4					6		1					3				5	
3 A	Helensville, 1M. North	23.1	10	A	F	DX	CM	4						2							1					3					
B	Helensville, 1M. North	23.1	10	A	S	DX		3													1							2			
4 A	Tuakau, 2M. North	7.3	9	A	F	D	CL		6				3	4	1		9				5		8			2				7	
B	Tuakau, 2M. North	7.3	9	A	U	D		4		6			5	3	1		7				2										
5 A	Waitoa, 4M. South	5.3	1	A	F	DX	CM	4	5				2		6						1					3				7	
B	Waitoa, 4M. South	5.3	1	A	S	DX		3					2								1										
6 A	Rotorua, Old Quarry Rd.	4.3	3	A	F	D	CM*						3		1						4					2					
B	Rotorua, Old Quarry Rd.	4.3	3	A	U	D								4	1													2		3	
7 A	Manaia, Taranaki	3.4	2	A	F	D	CL						1		5	2										3				4	
B	Manaia, Taranaki	3.4	2	A	S	D							1		2													3		4	
8 A	Gisborne, 10M. West	12.2	1	A	H	M	CSM						3		2											1					
B	Gisborne, 10M. West	12.2	1	A	S	M				1				3	6						2					4				5	
9 A	Sanson, Penny's Line	27.3	2	A	F	D	SL						3			1										2					
B	Sanson, Penny's Line	27.3	2	A	U	D							4	1		2						5					3				

10 A	Woodville, 8M. North	26.3	10	B	F	D	CSM					2									1				
B	Woodville, 8M. North	26.3	10	B	S	D							1	3	2										
11 A	Pukekohe, 4M. North	7.3	10	A	R	D	CM		4			5		2				3			1				
B	Pukekohe, 4M. North	7.3	10	A	S	D							1	6				3		5		4		2	
12 A	Otorohanga, 12M. South	7.3	4	A	R	D	CM		3			4		1				5			2			6	
B	Otorohanga, 12M. South	7.3	4	A	S	D							2	1	5			3				4		6	
13 A	Inglewood, 3M. East	2.4	5	B	F	DX	CM					2			1			4			3				
B	Inglewood, 3M. East	2.4	5	B	S	DX		4					1	3				5					2		
14 A	Mangaweka, 1M. South	18.3	2	B	R	MX	SM					3	4	5	2						1				
B	Mangaweka, 1M. South	18.3	2	B	S	MX						2		1					5		3			4	
15 A	Marton, Read's Road	27.3	3	A	F	D	CM					2	4	3	5						1				
B	Marton, Read's Road	27.3	3	A	U	D							2	1								3	4		
16 A	Masterton, 6M. South	25.3	12	A	F	M*	CS					2	1	3	4										
B	Masterton, 6M. South	25.3	12	A	S	M*						5	1	3	2							4			
17 A	Kaitaia, Aerodrome Rd.	16.1	4	A	F	DX	CM	3				2						1							
B	Kaitaia, Aerodrome Rd.	16.1	4	A	S	DX		2										1							R
18 A	Victoria Valley, Peria Rd	16.1	5	A	H	D	CM	4			5		2		3			1							
B	Victoria Valley, Peria Rd	16.1	5	A	U	D		2										1							BF
19 A	Punakitere, Kaikohe	19.1	4	A	F	MX	CM					3		4				1			2	5			R
20 A	Kaikohe, 3M. East	19.1	5	A	R	D	CM	5				4			7		2	1			3			6	
B	Kaikohe, 3M. East	19.1	5	A	S	D		3						2				1							
21 A	Whangarei, 13M. South	21.1	1	A	F	DX	CM					4		3				1			2				
B	Whangarei, 13M. South	21.1	1	A	S	DX						4		3				1			2				
22 A	Dargaville, 12M. North	22.1	11	A	F	MX	CM	2				5		4				1						3	
B	Dargaville, 12M. North	22.1	11	A	S	MX		3						5				1				2		4	
23 A	Dargaville, 5M. North	22.1	1	A	F	MX	CM	2		5	4	3						1							
B	Dargaville, 5M. North	22.1	1	A	S	MX		5						4				1				3	6	2	
24 A	Paeroa, 4M. North	5.3	2	A	F	DX	CM	5	6			2		4				1			3			7	
B	Paeroa, 4M. North	5.3	2	A	S	DX								2				1							
25 A	Whakatane, 3M. South	14.2	2	A	F	DX	CM					2						1							
B	Whakatane, 3M. South	14.2	2	A	S	DX						2				4		1				3			
26 A	Opotiki	14.2	9	A	F	MX	CM					2						1							
B	Opotiki	14.2	9	A	S	MX										2		1							
27 A	Victoria Valley School	16.1	1	A	F	MX	CM	2				3		5				1						4	
B	Victoria Valley School	16.1	1	A	S	MX		3						5				1				2		4	
28 A	Victoria Valley, Fryer's Rd.	16.1	4	A	H	D	CM	2				3	4					1							

								CLOVERS**						GRASSES**																		MISC.
STATION NUMBER A, Paddock; B, Roadside	LOCATION	DATE (1957)	TIME Nearest daylight hour	WEATHER A, Sunny; B, Cloudy; C, Strong Wind	TOPOGRAPHY F, Flat; H, Hilly; R, Rolling; S, Sealed; U, Unsealed rd.	SOIL MOISTURE D, Dry; M, Moist; W, Wet; X, Excessive at times	GRAZING C, Cattle; H, Horses; S, Sheep; L, Close; M, Medium	*Lotus* spp.	Red	Strawberry	Subterranean	Suckling	White	Browntop	Cocksfoot	Crested dogstail	Crowfoot	*Danthonia* spp.	Five-finger	Meadow foxtail	*Paspalum dilatatum*	*Phalaris* spp.	*Poa* spp.	Prairie	Ratstail	Rye	Sweet vernal	Tall fescue	Timothy	Yorkshire fog	B, Blackberry; F, Bracken fern; G, Gorse; R, Rushes; T, Titree
B	Victoria Valley, Fryer's Rd.	16.1	4	A	U	D															1										FR
29 A	Kerekere	18.1	10	AC	F	D	CM	5					4	3				2			1										G
B	Kerekere	18.1	10	AC	U	D												2			1										FG
30 A	Puketona, B.O.I.	18.1	9	AC	F	D	CM						3	2							1										R
B	Puketona, B.O.I.	18.1	9	AC	U	D								3	2						1										G
31 A	Wellsford, 7M. North	22.1	3	B	H	D	SM					5	4	2		6					1									3	
B	Wellsford, 7M. North	22.1	3	B	S	D															1							2			FG
32 A	Gisborne, 20M. North	12.2	3	A	H	D	SM						3	1		2											4				
B	Gisborne, 20M. North	12.2	3	A	S	D							4	2							1				3						
33 A	Te Matai, 1M. North	4.3	5	A	F	M	CL						2								1					3					
B	Te Matai, 1M. North	4.3	5	A	S	M								1							2				4					3	
34 A	Whangaroa, 5M. East	17.1	12	A	H	D	SM							2				1			5				4		3				
35 A	Whangaroa, 1M. East	17.1	1	A	H	D	CHL	3						2				4			1										
B	Whangaroa, 1M. East	17.1	1	A	U	D		3						2				4			1										
36 A	Pukemiro, 5M. South	7.3	1	B	H	D	SM							1		3		2													
37 A	Castlepoint, 3M. West	25.3	4	AC	H	D	CSM		4				3	2		5		1								6					
B	Castlepoint, 3M. West	25.3	4	AC	U	D							5	1	4	3		2													R
38 A	Porangahau, Blackhead Rd.	26.3	3	A	R	M	SM						3			2										1					

B	Parongahau, Blackhead Rd.	26.3	3	A	U	M					1	2												R
39 A	Tutira, 3M. North	11.2	6	A	R	D	CM			4	1	5		3					6				2	
B	Tutira, 3M. North	11.2	6	A	S	D					1	3		2		4							5	
40 A	Warkworth, 7M. South	22.1	5	A	H	D	CM	4		5	1					6					3		2	
B	Warkworth, 7M. South	22.1	5	A	U	D						2									1		3	F
41 A	Woodhill, 2M. South	23.1	12	A	R	D	CM				2	4		3		1								
B	Woodhill, 2M. South	23.1	12	A	S	D					3	2				1					4			
42 A	Foxton	4.4	2	AC	F	D	CM				1			2						3				
B	Foxton	4.4	2	AC	S	D					1			2						3				
43 A	Maraekakaho	11.2	12	A	R	D	CL			5	3			1	7			4		2	6			
B	Maraekakaho	11.2	12	A	S	D					3			1	6			4		2	5			
44 A	Norsewood, 4M. South	26.3	11	B	H	M	CM			2	1	3	6						5				4	
B	Norsewood, 4M. South	26.3	11	B	S	M				2	1	3	6						5				4	
45 A	Whananaki, 10M. West	21.1	12	A	H	D	CM	2		4	1			3					5					RT
B	Whananaki, 10M. West	21.1	12	A	U	D					2			1		3								T
46 A	Silverdale, 3M. West	23.1	10	A	R	D	CM	6		5	1					2			4				3	
B	Silverdale, 3M. West	23.1	10	A	U	D		3			1			2		4								FGT
47 A	Maramarua, 6M. East	6.3	4	B	R	DX	SM	7		8	1	6	4			2			3	9			5	
B	Maramarua, 6M. East	6.3	4	B	S	DX		3		6	1	4				2				5				
48 A	Te Kuiti, 4M. South	8.3	10	A	H	D	SM				5		2	1		3			6				4	
B	Te Kuiti, 4M. South	8.3	10	A	S	D						1	4			3					2			
49 A	Tarata	2.4	1	A	H	D	CSM			5	1	4	2			3		6		8			7	
B	Tarata	2.4	1	A	S	D					3	2									1			F
50 A	Hunterville, 4M. North	18.3	12	A	H	D	SM	9		4	1	6	2	8			10			3	7		5	
B	Hunterville, 4M. North	18.3	12	A	S	D		9		4	1	6	2	8			10			3	7		5	R
51 A	Carterton, 3M. North	25.3	1	B	F	M	CSM			4	1		2	3										
B	Carterton, 3M. North	25.3	1	B	S	M				3	1	2	4											
52 A	Eketahuna, 5M. South	25.3	11	A	R	D	CSM			3	1		2											
B	Eketahuna, 5M. South	25.3	11	A	S	D			5	6	1	3	2	4									7	
53 A	Eketahuna-Pahiatua, Brown's Road	25.3	6	B	F	D	CM			2			3						1					
B	Eketahuna-Pahiatua, Brown's Road	25.3	6	B	S	D				4	1	2								5		3		
54 A	Napier-Taupo, Mohaka R	15.2	3	A	R	D	CHM	4		2	1			3										
B	Napier-Taupo, Mohaka R	15.2	3	A	U	D				2	1	3		4									5	
55 A	Tiniroto, 5M. East	12.2	11	A	H	D	CSM			7	3	4		1		6		5	8				2	

STATION NUMBER A, Paddock; B, Roadside	LOCATION	DATE (1957)	TIME Nearest daylight hour	WEATHER A, Sunny; B, Cloudy; C, Strong Wind	TOPOGRAPHY F, Flat; H, Hilly; R, Rolling; S, Sealed; U, Unsealed rd.	SOIL MOISTURE D, Dry; M, Moist; W, Wet; X, Excessive at times	GRAZING C, Cattle; H, Horses; S, Sheep; L, Close; M, Medium	CLOVERS**						GRASSES**																	MISC.
								Lotus spp.	Red	Strawberry	Subterranean	Suckling	White	Browntop	Cocksfoot	Crested dogstail	Crowfoot	*Danthonia* spp.	Five-finger	Meadow foxtail	*Paspalum dilatatum*	*Phalaris* spp.	*Poa* spp.	Prairie	Ratstail	Rye	Sweet vernal	Tall fescue	Timothy	Yorkshire fog	B, Blackberry; F, Bracken fern; G, Gorse; R, Rushes; T, Titree
56 A	Waikohu, Opotiki	13.2	10	A	H	D	CSM											1			2				3						
B	Waikohu, Opotiki	13.2	10	A	S	D				2			4								1					3					
57 A	Bombay	23.1	2	A	R	D	CM						6	3		2					4					1				5	
B	Bombay	23.1	2	A	S	D		8					7	3	1			6			4					9		2		5	
58 A	Te Kauwhata	23.1	3	B	R	D	CM						4	5	3			7			1				8	2		9		6	
B	Te Kauwhata	23.1	3	B	S	D		6					8	3	5			7			1					2				4	
59 A	Horotiu, 2M. South	23.1	4	B	F	D	CM						2		3											1				4	
B	Horotiu. 2M. South	23.1	4	B	S	D		7					5	6	2						4				3					1	
60 A	Te Awamutu, 6M. South	23.1	6	A	F	D	CM						2		3											1				4	
B	Te Awamutu, 6M. South	23.1	6	A	S	D		5					6	4	2										3	8		1		7	
61 A	Te Kuiti, 11M. South	24.1	9	A	F	D	CM		5				4	6							1					3				2	
B	Te Kuiti, 11M. South	24.1	9	A	S	D			2	1				5							3									4	
62 A	Pio Pio	24.1	10	A	H	D	CSM						4	1				2			5						7	3		6	
B	Pio Pio	24.1	10	A	S	D			5					6	1						4							2		3	
63 A	Mokau, 5M. South	24.1	12	B	R	D	CM		5				4								2				6	3				1	
B	Mokau, 5M. South	24.1	12	B	S	D		6						5	2						3							1		4	
64 A	Uruti, 5M. South	24.1	2	A	F	D	SL					6	3	4		2					1					5					
B	Uruti, 5M. South	24.1	2	A	S	D		5							1						6				3			2		4	
65 A	Waitara, 8M. South	24.1	3	A	F	D	CL		4				2		5											1				3	
B	Waitara, 8M. South	24.1	3	A	S	D		2						3	1	4															

66 A	Eltham, 1M. South	24.1	4	A	F	D	CM		6		2		3	4							1			5	
B	Eltham, 1M. South	24.1	4	A	S	D			6		5	4	1	2							7			3	
67 A	Hawera	24.1	5	A	F	D	CM				5		1	3							2			4	
B	Hawera	24.1	5	A	S	D			4			3	1										2	5	
68 A	Waverley, 1M. North	24.1	5	A	F	D	SM				4			2							3			1	
B	Waverley, 1M. North	24.1	5	A	S	D					4		2	5									1	3	
69 A	Kai Iwi	24.1	6	A	R	D	CSM		4		3			2							1				
B	Kai Iwi	24.1	6	A	U	D					4		1	5									2	3	
70 A	Whangaehu, 2M. South	30.1	2	A	R	D	CSM				2	4	3								1			5	
B	Whangaehu, 2M. South	30.1	2	A	S	D					4	1	2											3	G
71 A	Bulls, 6M. West	30.1	3	A	F	D	SM		3		2	5		4							1				
B	Bulls, 6M. West	30.1	3	A	S	D					6	1	4				3				5		2		
72 A	Himatangi, 7M. North	30.1	4	A	F	D	SM					2	5								4	3		1	
B	Himatangi, 7M. North	30.1	4	A	S	D							2										1	3	FG
73 A	Levin, 5M. North	31.1	1	A	R	D	CM*				3		2								1	5		4	
B	Levin, 5M. North	31.1	1	A	S	D		3			4	2	1					6						5	
74 A	Otaki, 3M. South	31.1	3	A	F	D	CSL					1	2	4								3		5	
B	Otaki, 3M. South	31.1	3	A	S	D						1	2									4	3		
75 A	Paekakariki	31.1	4	A	F	D	CSL				2			3							1				
B	Paekakariki	31.1	4	A	S	D							2										1		
76 A	Norsewood, 2M. West	11.2	10	A	F	D	SM				3	1		2							4				
B	Norsewood, 2M. West	11.2	10	A	S	D					4	1	2	5								6		3	
77 A	Takapau, 2M. East	11.2	11	A	F	D	CM				3	1		2							5			4	
B	Takapau, 2M. East	11.2	11	A	S	D					4	1	2										3		R
78 A	Pakipaki, 1M. West	11.2	12	A	H	D	CSM				5		4	2	1									3	
B	Pakipaki, 1M. West	11.2	12	A	S	D					1	6	2	3	4				5						
79 A	Tutira Lakeside	11.2	5	A	R	M	CM				1		5	3							2			4	
B	Tutira Lakeside	11.2	5	A	S	M					5	1									4		3	2	
80 A	Wairoa, 3M. North	12.2	9	A	R	D	CM*				6	3				4	1			5			2		
B	Wairoa, 3M. North	12.2	9	A	S	D						3					1					4	2	5	B
81 A	Gisborne, 15M. West	12.2	12	A	H	D	SM				4	1		2			3					5		6	
B	Gisborne, 15M. West	12.2	12	A	S	D					3			6	1		2						5	4	
82 A	Gisborne, 10M. West	12.2	12	A	F	MX	CM				2						1				3				
B	Gisborne, 10M. West	12.2	12	A	S	MX				1	3	2	4				5				6				
83 A	Tolaga Bay, 1M. South	12.2	4	A	F	M	CSM				3	4					1				2				
B	Tolaga Bay, 1M. South	12.2	4	A	S	M		5			3	4					1				2				
84 A	Tolaga Bay, 5M. South	12.2	4	A	H	D	CM							3	1		2					4			

								CLOVERS**						GRASSES**																	MISC.
STATION NUMBER A, Paddock; B, Roadside	LOCATION	DATE (1957)	TIME Nearest daylight hour	WEATHER A, Sunny; B, Cloudy; C, Strong Wind	TOPOGRAPHY F, Flat; H, Hilly; R, Rolling; S, Sealed; U, Unsealed rd.	SOIL MOISTURE D, Dry; M, Moist; W, Wet; X, Excessive at times	GRAZING C, Cattle; H, Horses; S, Sheep; L, Close; M, Medium	*Lotus* spp.	Red	Strawberry	Subterranean	Suckling	White	Browntop	Cocksfoot	Crested dogstail	Crowfoot	*Danthonia* spp.	Five-finger	Meadow foxtail	*Paspalum dilatatum*	*Phalaris* spp.	*Poa* spp.	Prairie	Ratstail	Rye	Sweet vernal	Tall fescue	Timothy	Yorkshire fog	B, Blackberry; F, Bracken fern; G, Gorse; R. Rushes; T, Titree
B	Tolaga Bay, 5M. South	12.2	4	A	S	D	CM											2			1						3				
85 A	Matawai, 3M. West	13.2	11	A	R	MX	CM						2	1	5	6										4				3	
B	Matawai, 3M. West	13.2	11	A	U	MX							2	1	7	5		3								6				4	
86 A	Opotiki, 12M. West	14.2	10	A	H	D	CM						2	7	4	3		5			1					6					
87 A	Opotiki, 20M. West	14.2	11	A	R	D	CM						2		4						1					3					
B	Opotiki, 20M. West	14.2	11	A	S	D		4					5	2							1									3	
88 A	Te Pohue	15.2	4	A	H	D	CSM	4					3	1				2									5				
B	Te Pohue	15.2	4	A	S	D		5						1	3			2									6			4	
89 A	Turangi, 10M. South	4.3	12	A	R	D	CM						2	3	5			1												4	
B	Turangi, 10M. South	4.3	12	A	S	D			5					2	4			1												3	
90 A	Taupo, 12M. North	4.3	1	AC	F	M	—*		3				4		1	2														5	
B	Taupo, 12M. North	4.3	1	AC	S	M			1						2															3	
91 A	Rotorua, 25M. South	4.3	2	A	F	D	CM						2	3	7									6		1			5	4	
B	Rotorua, 25M. South	4.3	2	A	S	D			7				6	4	1									2		5		3			
92 A	Okere Falls, 4M. North	4.3	4	A	R	D	CM						2		3						4					1					
93 A	Tauranga, 6M. West	5.3	10	A	F	D	CM						2								1					3					
B	Tauranga, 6M. West	5.3	10	A	S	D						5	4	3	2						1										
94 A	Kaimai	5.3	10	AC	H	D	CSM							1				3			2										
B	Kaimai	5.3	10	AC	S	D		4						1				2			3										
95 A	Morrinsville, 13M. South	5.3	12	A	F	M	CM						3								1					2					

B	Morrinsville, 13M. South	5.3	12	A	U	M			3				4	2			1										
96 A	Whitianga	6.3	10	A	F	M	CM				7	6		4	2		1				5	3					
B	Whitianga	6.3	10	A	U	M		3						1			2										
97 A	Manaia, Coromandel	6.3	12	B	F	D	CM					3					1					2					
B	Manaia, Coromandel	6.3	12	B	S	D											1										B
98 A	Hikateruru, 3M. North	6.3	3	A	F	D	CL	3				5	2				1					4					
B	Hikateruru, 3M. North	6.3	3	A	S	D		5					2	4			1		3								
99 A	Tangitu, 12M. North	8.3	10	B	R	D	CSL	8			7	6	1		4							3	5			2	
B	Tangitu, 12M. North	8.3	10	B	S	D								2	4				1							3	
100 A	Taumarunui, 8M. South	8.3	1	B	R	D	CL					3	1	5	4	6						7	8			2	
B	Taumarunui, 8M. South	8.3	1	B	S	D							1	6				4		3			5			2	
101 A	Taihape, 6M. North	18.3	3	A	H	M	SM					2	4		1	3											R
B	Taihape, 6M. North	18.3	3	A	S	M						1	5	2	4			6	7							3	
102 A	Hihitahi, 1M. North	18.3	4	BC	R	M	CHM						1		3	2							5			4	
B	Hihitahi, 1M. North	18.3	4	BC	S	M			3			4	1										5			2	
103 A	Ohakune, 12M. East	18.3	4	BC	F	M	SM*					2		1								3				4	
B	Ohakune, 12M. East	18.3	4	BC	S	M						3	1			2										4	
104 A	Ohakune, 3M. East	18.3	4	B	R	M	—*		1			2	4									3				5	
B	Ohakune, 3M. East	18.3	4	B	S	M			3			4	1	2												5	
105 A	Palm. N. Pahiatua Track	25.3	9	BC	H	M	SM					5	1	4	3	2										6	
B	Palm. N. Pahiatua Track	25.3	9	BC	U	M						5	1	4	3	2										6	
106 A	Masterton, 7M. North	25.3	11	A	F	M	CM					2	4		3							1					
B	Masterton, 7M. North	25.3	11	A	S	M			3			4	2	1					5								
107 A	Greytown, 2M. West	25.3	1	A	F	M	CM					2		3								1					
B	Greytown, 2M. West	25.3	1	A	U	M						3	1	2											4		
108 A	Taueru, 2M. North	25.3	2	BC	R	M	SM					3	4		2							1					R
B	Taueru, 2M. North	25.3	2	BC	S	M				7		2	1	3	5										6	4	
109 A	Mangapakeha, 8M. East	25.3	4	B	F	M	SM					1										2			3		
B	Mangapakeha, 8M. East	25.3	4	B	U	M				1					3							2		4			
110 A	Porangahau	26.3	2	BC	F	M	CM					2			3							1					
B	Porangahau	26.3	2	BC	U	M						3		2					1	4				5			
111 A	Wallingford, 5M. North	26.3	4	BC	H	M	SM					3	1		2												R
B	Wallingford, 5M. North	26.3	4	BC	U	M							1	3	2									4			
112 A	Hunterville, 6M. South	27.3	5	A	F	M	SM					3	1		2							4					
B	Hunterville, 6M. South	27.3	5	A	S	M							1	2											3		
113 A	Kohinui, 5M. East	28.3	5	A	H	M	CSM	6				2	4		1							3	5				
B	Kohinui, 5M. East	28.3	5	A	U	M						3	1	2													F

---

* Pasture cut recently for hay.

** Numbered in order of dominance.

HOUSING A GUINEA PIG COLONY

By I. J. CUNNINGHAM, Animal Research Station, Department of Agriculture, Wallaceville

(*Received for publication, 11 June 1958*)

Summary

A description is given of a system of managing a guinea pig colony so as to use labour and foodstuffs as efficiently as possible.

INTRODUCTION

Two main methods have been used to house guinea pigs, the "floor method" and the "cage method". In the floor method the guinea pigs are run in groups of about 30 on a floor of concrete or asphalt covered with a litter of sawdust, and the litter is renewed once or twice each week. In the cage method, the guinea pigs are run in smaller groups in cages with wire mesh floors which have trays beneath on to which waste matter falls.

The floor method has an advantage over the cage method in requiring less labour for cleaning and feeding, and this is important since wages represent about half the running costs of a small animal colony. A disadvantage is that the guinea pigs and their food are in close contact with excreta so that good hygiene and disease control are difficult to maintain. Though labour costs in cleaning and feeding are low this is offset to some extent by a surprisingly high cost of dry sawdust, and by losses of food through fouling or being buried in the litter.

The cage method gives improved hygiene but labour costs are higher. Feeding and cleaning and handling guinea pigs through cage doors is slow and the cleaning of trays from beneath the cages is especially time consuming.

An intensive cage system has been devised by Lane-Petter (1954) in which guinea pigs are kept in batteries of four tiers of cages. Between each tier of cages is a tray covered by bituminised paper, and the paper can be replaced by pulling through from a roll fixed at one end. Cleaning is simply accomplished by pulling through and discarding the paper. This system of housing has many advantages in saving of space and labour.

The system recently adopted at Wallaceville Animal Research Station is described below as it incorporates the best features of the methods mentioned and adds some new ones which further simplify and cheapen the maintenance of the guinea pig colony.

THE CAGE

Figure 1 shows a photograph of the cage and a scale drawing to give the dimensions. The cage encloses an area of 6½ feet by 2 feet and

N.Z. J. agric. Res. 1: 750–756.

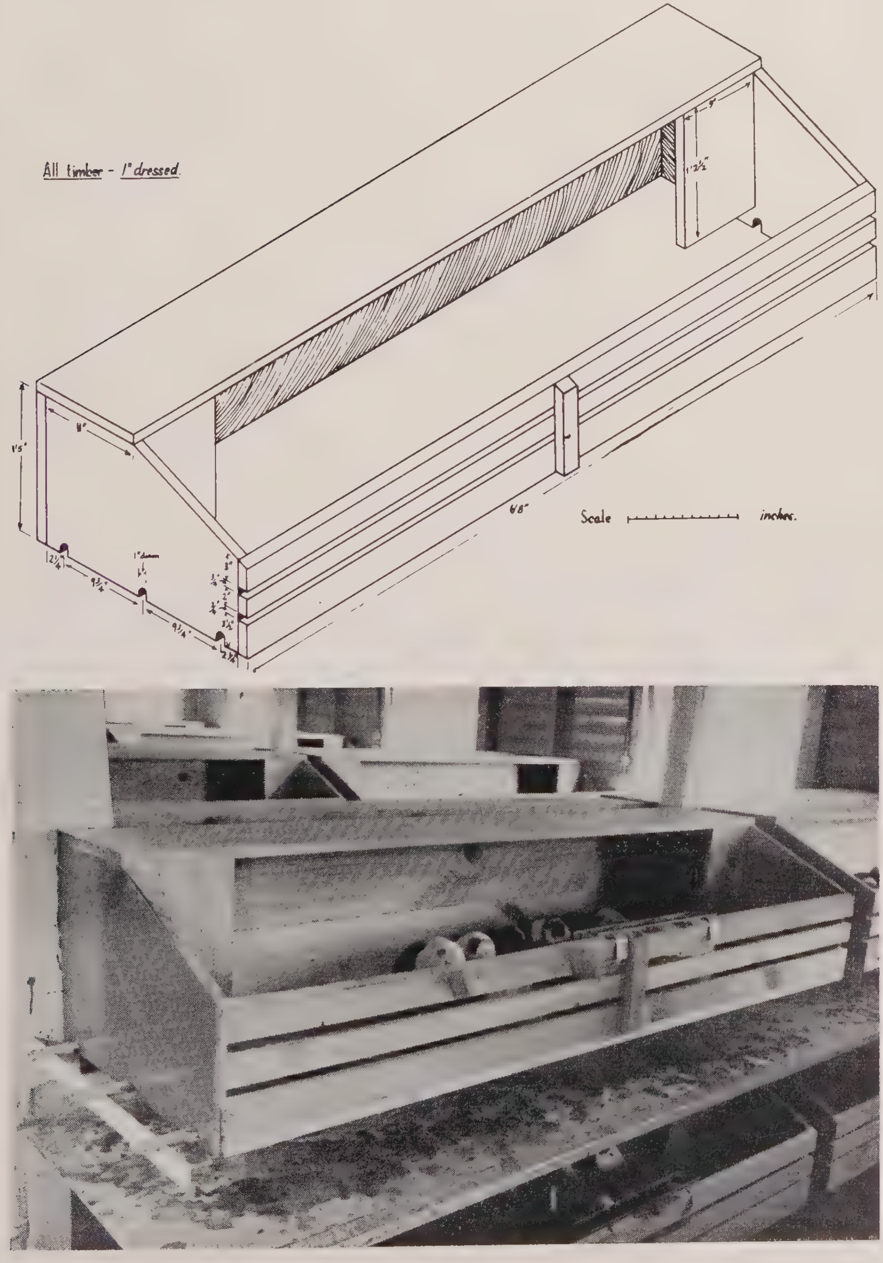

All timber - 1" dressed.
9"
1'2½"
1'5"
11"
6'8"
Scale
inches.
2¼
9¼"
1" diam
1¼
9¼"
2¼
3"
¼"
2"
¼"
1½"

Fig. 1.—Photograph and scale drawing of guinea pig cage.

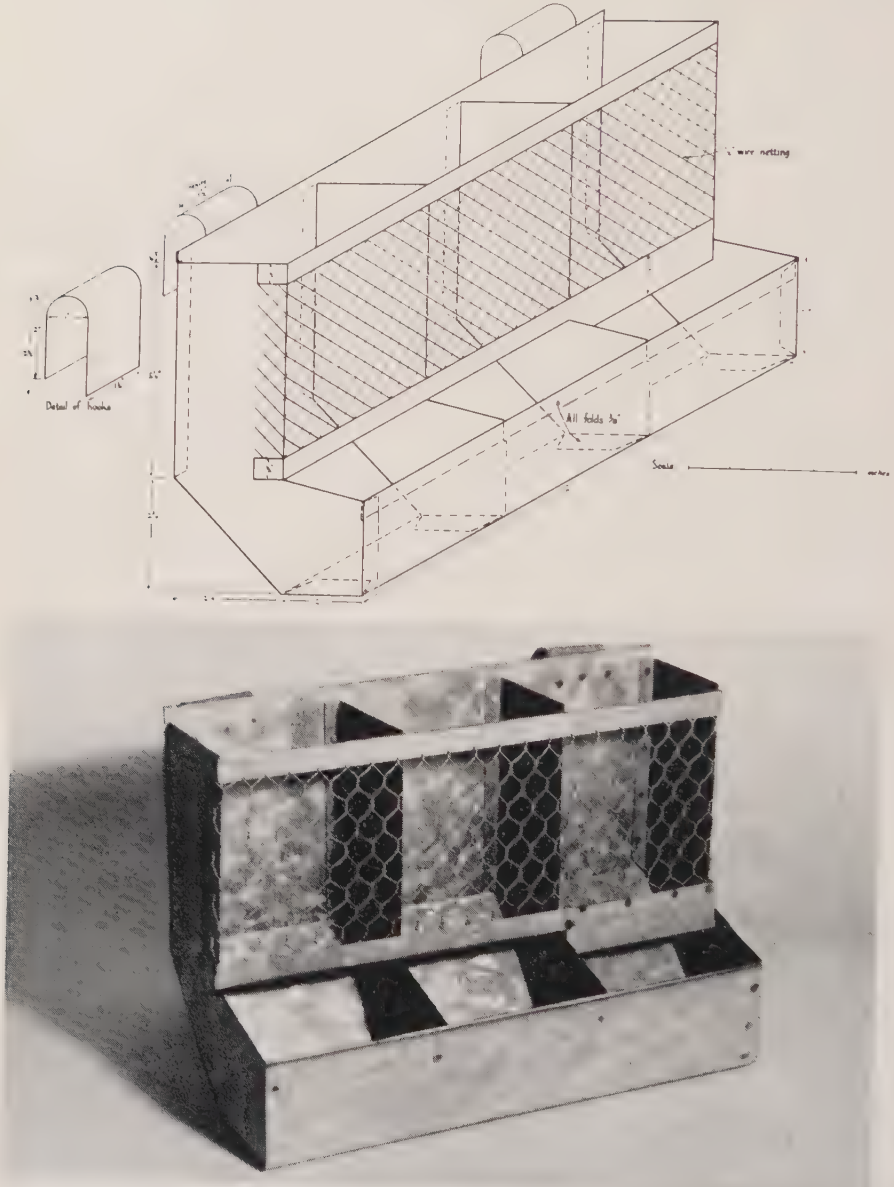


Fig. 2.—Photograph and scale drawing of food hopper.

is made of wood. The wooden cage is supported on three bars made of ½ in. galvanised-iron water pipe, and resting on top of these bars and fitting closely inside the cage is a wire mesh floor made of crimped 14 gauge wire, woven so as to leave spaces 1 inch by ¼ inch. Inside the cage are two baffle plates which form havens for young animals at each end of the cage. The top of the cage is not closed by a door and it is found that guinea pigs rarely escape. Dispensing with a door cheapens construction and facilitates feeding and handling. The separate floor makes cleaning easier.

The cages were made from wood to save cost. Wooden cages of the design shown and without a floor were made for £2/17/6d, while cages made to a similar design in 24-gauge galvanised iron cost £6/17/6d. The wire floor costs £2/5/-.

THE FOOD HOPPER

The food hopper was planned to hold pelleted food, chaffed hay, and lawn clippings or long grass, in three separate compartments. Figure 2 shows a photograph and a scale drawing of the design reached after a number of trials. Hoppers of two sizes are used, the 12 in. one which is depicted, and a larger size 18 in. long which is used for fully stocked cages. The angle of the floor is important to allow foodstuffs to move from the vertical compartment to the horizontal chamber, and the level of the bottom of the front panel is critical so as to allow movement down of foodstuffs and yet not permit too rapid movement and overflowing. The opening below the lawn clippings compartment has to be smaller to reduce loss. Guinea pigs are particularly fond of grass clippings and when these are present in the hopper they scuffle round and cause overflowing. The half inch wire netting front was found necessary so that if there was a blockage of any of the foods the guinea pigs could nibble through the netting and release the blockage. With such a front, moreover, longer grass, which would not slip down to the bottom, could be eaten through the openings of the netting. The hopper is thus suitable for all of the foodstuffs employed except green vegetables and roots, and these are thrown on to the floor of the cage.

The hopper was made of 24-gauge galvanised iron and $\frac{1}{2}$ inch galvanised wire netting and joints were spot welded.

WATER

Water is supplied in glass or metal jars about $3\frac{1}{2}$ in. diameter and 4 in. high. It has been found that this is a clean and satisfactory method as no contamination occurs and in most cases the jar is not overturned. In the odd cage where the guinea pigs acquire the habit of overturning the jar this is secured by a wire.

The advantage of an open jar is readiness with which the water can be replenished through a valve at the end of a flexible hose, and ease of cleaning the container. Both of these attributes are regarded as essential. The inverted bottle and glass drinking spout commonly employed for small laboratory animals was found to require too much time for dismantling and reassembling at each filling, and for periodic cleaning.

Attempts were made to supply water from containers with a valve at the bottom operated by the guinea pig when it drank. Several types were tried but none proved satisfactory as in every case there were leakages, which meant that animals in some cages would run short of water unless frequent inspections and replenishments were made. The leaked water also added to the difficulties of cleaning.

CAGE ARRANGEMENT

The cages are arranged in two tiers, both of which can be serviced by attendants standing on the floor. Any greater number of tiers caused loss of time and efficiency through having to bend to attend to lower tiers or mount ladders for the higher tiers. The wire floor of the bottom tier of cages is 20 inches from the floor of the building and waste from this lot of cages falls directly on to the floor, which has a covering of sawdust or shavings just below the cage to absorb urine and keep down smell.

The second tier of cages is directly above the lower lot and 30 inches higher. Eight inches below the floor of the top tier is a galvanised iron tray covered with paper on to which waste matter falls from the cages.

A satisfactory arrangement is to have cages arranged in bays on either side of a central service corridor.

A view of a breeder room is shown in Fig. 3.

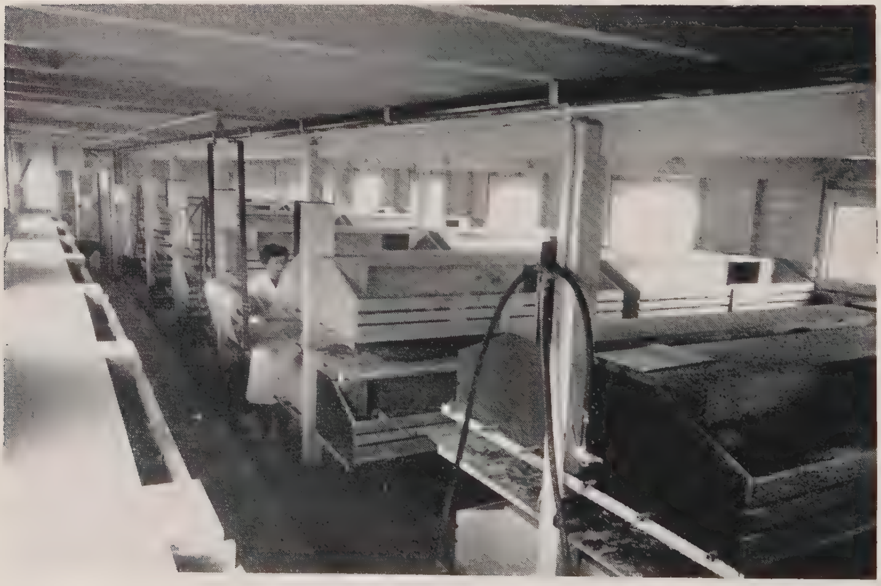


Fig. 3.—View of a breeder room.

CLEANING AND FEEDING ROUTINE

Cleaning

The trays and the floors are cleaned three times a week. The most economic paper to use on the trays has been found to be a medium-heavy brown grade (Sandow 80 lb). A sheet sufficient to cover the tray beneath two cages costs eightpence. Newsprint is cheaper when ends of rolls are bought, but this advantage is lost through added time taken in cleaning because of the breaking of the soft paper. Bituminised papers such as building paper or sisalcraft are very many times dearer. A convenient method of dispensing paper is by mounting the roll on a frame carried on a Henderson's track so that the frame can be moved along as required (see Fig. 3).

Attempts to collect waste on trays with no covering of paper, but to wash down with a jet of water, did not prove satisfactory because of the time taken to hose down and collect the mass of waste material. The quantity involved was too great to pass into the drainage system.

The cages are dismantled and the wire-mesh floors and supporting bars washed about once in three months.

Feeding

Food consists of pelleted meals, green food, and hay. The composition of the pellets has not yet been finally decided upon and is still under investigation for nutritional adequacy, and for suitable consistency of the pellets. It is essential that the pellet be hard and that a minimum of crumbling occurs when the guinea pigs gnaw at them. All powdered food is lost either by falling through the wire mesh of the floor or by refusal of the guinea pigs to eat powder from the hopper. The formula at present in use is as follows and the pellet is cylindrical, $\frac{3}{4}$ inch diameter and about 1 inch long.

	lb		lb
Lucerne meal	200	Liver meal	10
Ground wheat	700	Dried skim milk	320
Ground hulled oats	80	Calcium carbonate	20
Ground clipped barley	200	Common salt	10
Ground maize	120	Zinc oxide	70 grammes
Meat meal	320	D-L α tocopherol acetate	15 grammes
Dried blood	20		

Green feed is supplied as lawn clippings at the times of the year when these are available. Feeding in the hopper is necessary as otherwise the fine grass clippings are lost through the floor of the cage. When lawn clippings are not available, as in winter or in the dry summer, specially grown green crops or long grass are provided. The long grass can be fed in the hopper, but crops are placed on the floor of the cage. The crops grown are silver beet, rape, and soft turnips for summer use, and silver beet, swede turnips, chou moellier and thousand-headed kale for winter use. Guinea pigs prefer the leafy brassicas.

Hay is regarded as an essential feed but could not be supplied in the long state without considerable loss through the floor of the cage, or without fouling up the floor of the cage. This difficulty was overcome by chaffing the hay and feeding in one compartment of the food hopper.

Feeding chaffed hay, green food, and pellets in the hopper has proved the most economical of the methods tried in the labour required and in minimising waste of foodstuffs. The method also gives minimum contamination of cage through excess food lying about.

CARRYING CAPACITY

When used for breeding animals each cage holds 12 does and one buck kept on the post-partum mating system (Rowlands 1949). This density gives adequate space for the adult animals and their young up to weaning age. Unmated guinea pigs can be successfully kept at twice the density.

In an ideal arrangement with central corridor and cages on each side, 1200 does and 100 bucks or 2,600 unmated guinea pigs could be run in a building 30 feet by 50 feet.

In a heavily populated room there is considerable formation of ammonia which makes conditions unpleasant for attendants, and probably also for the guinea pigs. This is particularly true in cold weather when ventilation is kept down to reduce the demands for heating. The ammonia nuisance is overcome by spreading a thin layer of a mixture of 5% by weight of superphosphate in sawdust on paper beneath the cages; straight superphosphate, which has a pH of about 3.0 has been used and effectively absorbs the ammonia or prevents its formation. The mixture is spread thinly at the rate of about one pound per 7 square feet over the paper on the trays and on a layer of paper placed on the floor beneath the lower cages. End-of-roll newspaper is the most economical paper for the floor. If desired, the cleanings containing the superphosphate can be composted and used on the garden which provides green vegetables for the colony.

ACKNOWLEDGEMENTS

Acknowledgement is made to Mr. A. L. Bryant for preparation of the drawings and the photographs.

REFERENCES

LANE-PETTER, W. 1954: *J. Hyg. 52:* 151-4.

ROWLANDS, I. W. 1949: *Ibid. 47:* 281-7.

PHYSICAL AND CHEMICAL CHANGES IN THE DEVELOPING HOP CONE

By H. O. Askew, R. J. Monk, and Joyce Watson,
Cawthron Institute, Nelson

(*Received for publication, 16 May 1958*)

Summary

Data for dry-matter contents, minerals, nitrogen (total and protein), glucose, fructose, sucrose, starch, pentosans, solids soluble in petroleum ether, and alpha-resins, covering the four seasons, 1955 to 1958, are presented for hop flowers and developing cones.

Accretion of dry matter, minerals, nitrogen, pentosans, and solids soluble in petroleum ether, was continuous during growth of the cone; the concentration on the dry-matter basis of calcium, magnesium, potassium, phosphorus, and nitrogen steadily decreased once the cones had been formed.

Starch and sugars concentrations passed through distinct maxima, which occurred in the early stages of development of the cones to decrease to very low values at maturity. Soluble solids, alpha-resins and pentosans concentrations increased progressively with development until maturity, after which the first two constituents decreased. The amounts of starch and sugars per cone varied with development, and tended to decrease as the resins and pentosans increased.

Although seasonal conditions doubtless affected the levels of given constituents at a specified stage of growth, yet the same pattern of variation in these levels during development was found in each season.

INTRODUCTION

The results of an investigation on certain chemical changes, both inorganic and organic, in the chemical composition of developing hop cones was published in 1954 (Askew, Monk, and Hodgson). In that paper the material for analysis was obtained from the Hop Research Station at Riwaka. Each season since then investigations on somewhat similar lines have been carried out using Californian variety hops grown at the Marsden Research Farm at Wakefield, about 18 miles south of Nelson city.

The main points of interest in the present investigations have been the variations in dry matter, sugars, starch, pentosan, and resin contents. In the first investigation referred to above it was found that the reducing sugars varied greatly and that, in particular, over a certain relatively short period of time these sugars increased rapidly and then decreased just as rapidly. It was important therefore to confirm these changes and to study variations in other constituents over the period of development of the cone from the stage of the minute flower buds, through burring (flowering period) and gradual appearance of recognisable cones, to the fully mature cone at time of harvest, and even to the period of over-maturity. The present paper contains the results of these investigations over the four seasons 1955 to 1958.

N.Z. J. agric. Res. 1: 757–777

EXPERIMENTAL

The Californian variety of hop used in these experiments was grown on Motupiko silt loam, where a complete fertiliser at 10 cwt per acre had been applied annually. Ten hills, chosen for uniformity, provided the sample material. In the several seasons, sampling began at different stages of growth of the cones: thus in January 1956 the first samples consisted of minute flowers 2–3 mm in diameter so that 2800 were taken to form the sample. Later in that season, and in other seasons, the numbers of flowers or cones were gradually reduced, but at no time, even with fully developed cones, were less than 600 taken per sample. Fresh and dry weights were determined in every case and were later converted to a per-cone basis. Percentage of dry matter was calculated and weights of the various constituents present were converted to milligrammes of the constituent per cone.

Total ash was determined following ignition of the sample in an electric muffle furnace at approximately 450°C, and insoluble ash as the residue remaining after extraction with dilute hydrochloric acid of the acidified ash, which had been evaporated to dryness and gently baked. Calcium, magnesium, potassium, phosphorus, and total nitrogen were determined by methods already reported (Askew, Monk, and Hodgson, 1954). Protein nitrogen has been taken as nitrogen in compounds insoluble in water at 80°C. Sugars have been determined as (1) total reducing sugars, by the method of Somogyi (1945), (2) "glucose", as those sugars reacting with hypoiodite, (3) "fructose", as the difference between total reducing sugars and glucose, and (4) "sucrose", by the increase in reducing sugars following mild hydrolysis with acid. It is recognised that sugars other than these may be included, or omitted, in the estimations performed. Alpha-resins have been determined by the method of Ford and Tait (1932), and total soluble solids by solution in petroleum ether (b.p. 40–60°C). It is probable that some of the substances extracted from flowers and very young cones may not be resins as defined in the previous sentence. Starch was estimated by the method of Pucher, Leavenworth, and Vickery (1948), involving precipitation of the starch-iodine complex, and pentosans by the A.O.A.C. method (1945).

All analytical data are expressed on the dry-matter basis.

RESULTS

Physical Changes

The earliest stages for sampling were used in 1956, when the season's work began on 17 January with the collection of minute flowers, with some coming into burr. At this time the fresh weight per flower was 19 mg and the dry matter content 19.9%. Growth was small during the next eight days, the corresponding sample obtained on 25 January weighing 31 mg, with a dry-matter content of 22.3% (Fig. 1), the maximum figure for this series of samples. From then onwards growth was rapid, the sample of 6 February weighing 335 mg per cone and having a dry matter content of 14.5%. At this time the sample was composed of definitely formed cones, some being of appreciable size. It is interesting to note in Fig. 1 that although the dry-matter content of the developing cones passed through a maximum and then fell rapidly, the accretion of dry matter per cone was very regular. Indeed, beginning at 21 January, the dry matter is doubled every 5 days, rising from 5 mg per cone to 10 mg on 26th, to 20 mg on 31st, and to 40 mg on 5 February.

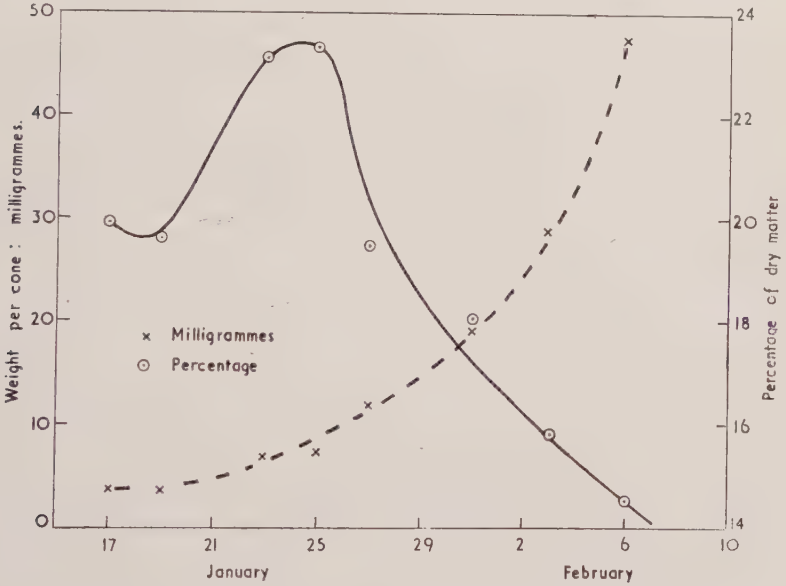


Fig. 1.—Percentage of dry matter, and weight of dry matter in milligrammes per cone, for very young cones, 1956 season.

The above data for 1956 have been incorporated in the graphs of variation of percentage of dry matter from minute flower and cone to the fully matured cone (Fig. 2), and of increase in dry matter per cone during growth over the same period (Fig. 3). In 1955, sampling began when the plants were in maximum burr and thus the earlier samples covered stages of growth a little previous to that of the last sample of 1956. In 1957, the samples covered approximately the same period of development, but in 1958 sampling was begun at the early burring stage and carried on to a period about 10 days before harvesting began. On 3 February 1955 the fresh weight per cone was 95 mg with dry matter content of 18.7%. A minimum dry matter content of 15.1% was found for the sample taken on 10 February when the average fresh cone weighed 685 mg. From Figs. 2 and 3 it can be deduced that the fresh weight increased from 865 mg on 21 February to 1060 mg for the mature cones harvested on 21 March. Over this period the percentage of dry matter increased to 24.1. In 1957 the minimum percentage of dry matter was 12.0 on 11 February, when the fresh weight was 750 mg per cone, and the dry weight 89.8 mg. As shown in Figs. 2 and 3 both percentage of dry matter and weight per cone increased at the end of the sampling period to figures appreciably greater than those of 1955.

In 1958 sampling began on 28 January at a time when the dry-matter content of the minute cones must have been at about its maximum, the figure on this date being 24.5%. This fell to a flat minimum of approximately 11.8% over the period 10-14 February. On 28 January the average weight per cone was 6.7 mg, which rose to 32.7 mg on 6 February, to 99.2 on the 12th, to 126.3 mg on the 17th, and to 156.2 mg on the 21st. Thus, between 28 January and 17 February the cones doubled their dry weight every five days, a relationship identical with that found for the 1955 series of samples. Examination of Figs. 2 and 3 containing data for different periods of sampling over the four seasons show that if allowance is made for seasonal effects the curves superimpose fairly well, and thus allow a reasonably close estimate to be made of the dry-matter content and percentage of dry matter on a given date, at any rate up to about 20 February. This would seem to indicate that in a late season, as in 1958, the cones develop very rapidly once burring has been completed, so that by the usual date for commencing harvesting, namely the end of February, the cones are at about the same stage of physical development from season to season, but, as Fig. 3 shows, growth from then on may be appreciably different.

Chemical Changes

Data for the ash, and individual elements, for samples taken in 1955 are not given in this paper because they followed somewhat the data given for these constituents in the original paper describing work on the growth of hop cones (Askew, Monk, and Hodgson, 1954). It has not been thought necessary to determine these constituents in the 1958 samples: therefore only the data for 1956 and 1957 will be considered, those for the former season because of the very early stages of

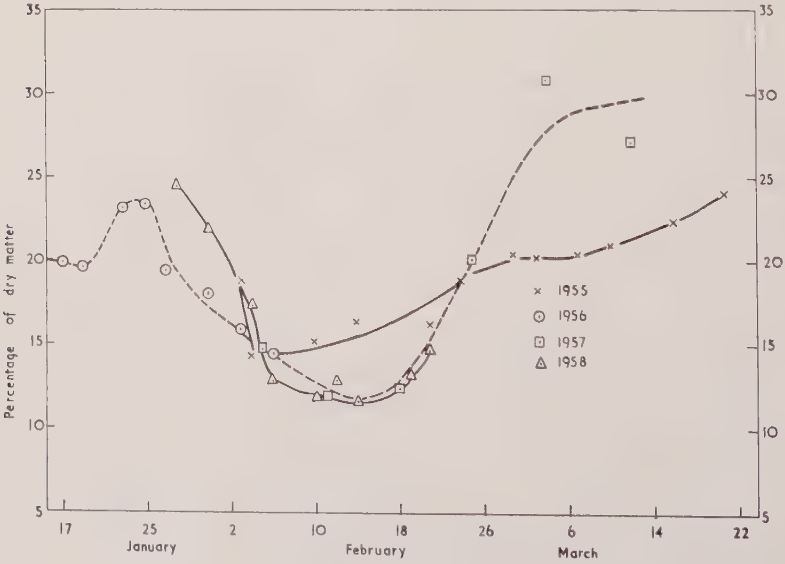


Fig. 2.—Variation in percentage of dry matter in developing cones.

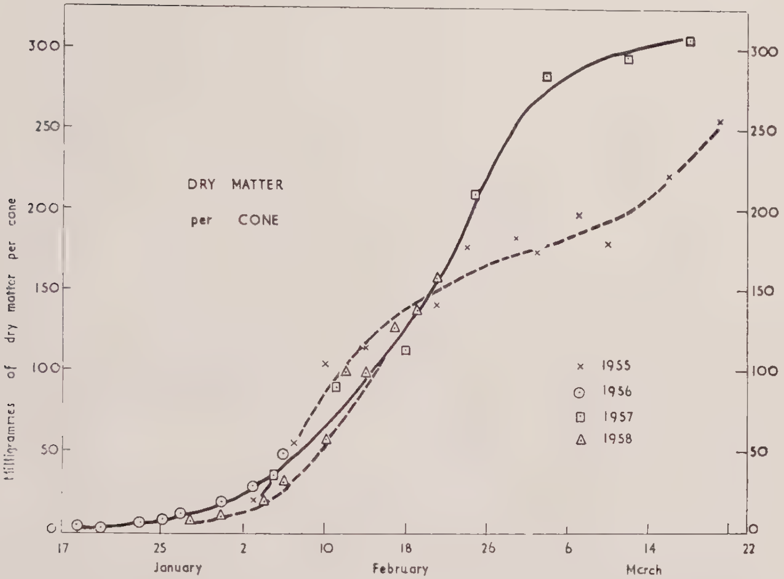


Fig. 3.—Accretion of dry matter in milligrammes per cone during growth.

growth that were dealt with, and those for the latter because the sampling period began at a growth stage similar to that at which the 1956 season ended. Data for nitrogen content are however available for the samples from all four seasons.

Mineral and Nitrogen Contents

Data for the soluble ash (total ash less insoluble ash) and for the insoluble ash contents of the dry matter of samples taken in 1956 and 1957 are given in Fig. 4. In the very small cones (January period) there are large fluctuations in both soluble and insoluble ash contents, the maximum figure for the former being 8.63% on 25 January, and for the latter 2.15% in the first sample of 17 January when the young flowers were not yet in burr. Small cones were present, however, by 25 January. With continual growth both soluble and insoluble ash contents fell appreciably until 6 February, when sampling ceased in 1956. At this time some of the cones were of reasonable size, giving soluble and insoluble ash contents of 7.37% and 0.49% respectively. In 1957, soluble ash was 8.61% and insoluble ash 0.80% on 5 February when sampling began. After this date the soluble ash decreased greatly and then fluctuated appreciably to finish the season at 7.74%. Over the same period the insoluble ash content varied only from 0.72% to 1.15%, this higher figure occurring in the last sample, taken on 18 March.

Results of determinations of calcium (as CaO), magnesium (as (MgO), potassium as (K_2O), and phosphorus (as P_2O_5) are given in Fig. 5. Calcium content was relatively low on 19 January (0.84% CaO) when potassium and phosphorus contents were relatively high.

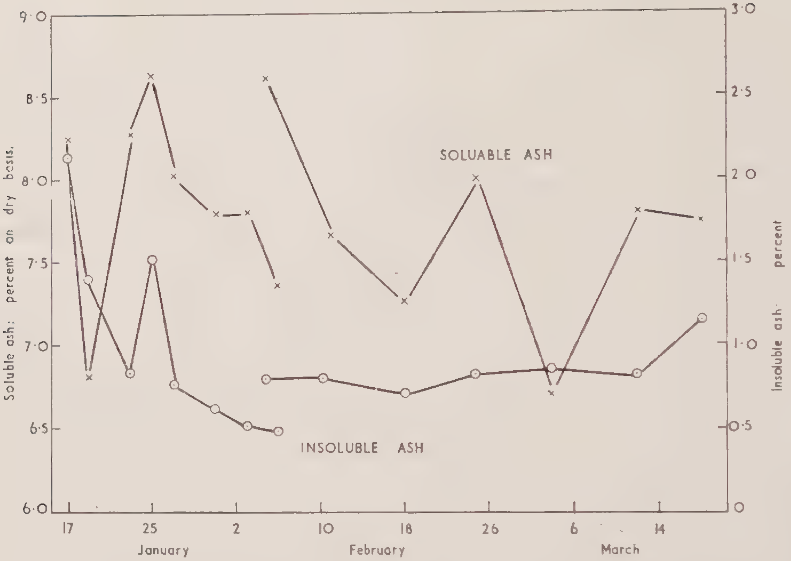


Fig. 4.—Soluble and insoluble ash contents of developing cones, 1956 and 1957 seasons.

Marked increases in calcium content followed, to the maximum figure of 1.58% CaO on 25 January when the sample consisted of flowers in burr, together with some small cones. Potassium content moved inversely to calcium over this period. Subsequently the calcium content decreased but the potassium content increased as the cones grew. Phosphorus content decreased steadily from the maximum figure of 2.02% P_2O_5 on 19 January to 1.11% when sampling finished on 6 February; at this time the cones were well-developed. Magnesium content increased slightly with growth until 25 January after which it decreased from the maximum figure of 0.75% MgO to 0.63% for the final sample. Making allowance for possible seasonal effect the data for the final sample of the 1957 season are in conformity with the data for the final sample of 1956. In the first sample of 1957 were flowers just past burring and some cones about one inch long. With growth the four constituents under discussion tended to decrease steadily until the end of the sampling period on 18 March, at which time the cones were over-mature. The final figures were 1.33% CaO, 0.57% MgO, 2.96% K_2O, and 0.96% P_2O_5, all of which are very similar to those reported previously (Askew, Monk, and Hodgson, 1954) for material from the same variety of hop but grown on a different soil type.

Data dealing with nitrogen are shown in Fig. 6. The percentages in the dry matter of flowers and cones are given for the samples collected in the four seasons 1955 to 1958. As has already been stated, in 1955 sampling began at the time of maximum burr; at this stage the nitrogen percentage was 4.4. This fell to a flat minimum of 2.6%, and then increased slightly towards the end of the season. For the minute flowers of 1956 a maximum figure of 6.17% was found on 19 January.

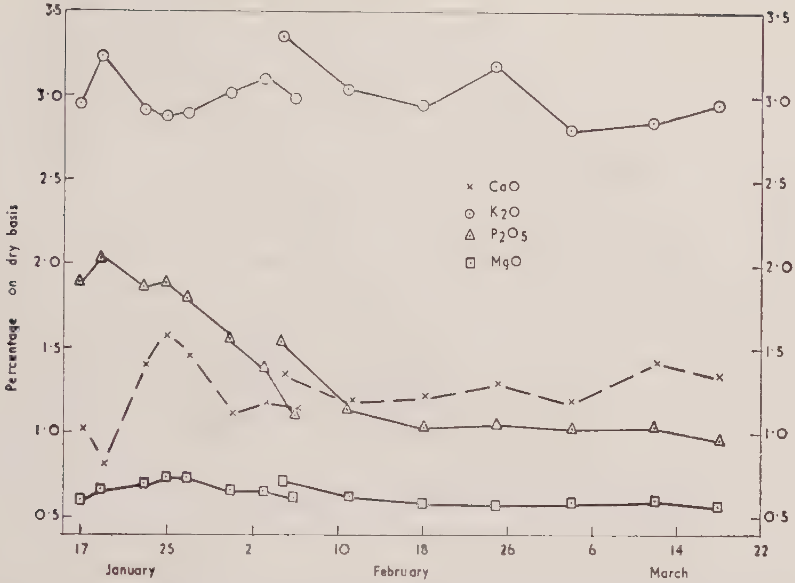


Fig. 5.—Calcium, magnesium, potassium and phosphorus contents of the dry matter of developing cones, 1956 and 1957 seasons.

The nitrogen content decreased rapidly to 3.58% on 6 February when the sample was composed of well formed cones. The curve of the data of 1956 fits very satisfactorily on to that of 1955, due doubtless to the first samples of 1955 being at a stage of development similar to the last ones of 1956. In 1957 it is evident that sampling began somewhat after the stage corresponding to maximum nitrogen content. However there is a rapid fall as in the two previous seasons, followed by a long period of slowly decreasing nitrogen content to a final figure of 2.60%. For the 1958 season the period of sampling has covered the high nitrogen range, the maximum figure in this season being 6.29%; this is very close to the maximum figure of the 1956 season. As in previous seasons the amount of nitrogen in the dry matter rapidly decreased as the cones grew in size. Making allowance for an apparent seasonal delay of about a week in 1958 it is clear that the curve for the data of this season would be superposable on the curves for the 1955 and 1956 seasons. A very constant pattern of variation in nitrogen content with development is therefore shown by the hop cones.

Further information on the changes taking place in the developing cone has been sought by determining the percentages for protein (hot-water insoluble) nitrogen of samples of the 1955 and 1958 seasons. These, together with the figures for total nitrogen, are shown graphically in Fig. 7.

In both years the pairs of curves are approximately parallel, especially later in the sampling periods. When expressed as a percentage of the total nitrogen the protein nitrogen falls from 69.7 for the first sample of 1955 at a fairly steady rate, except for the aberrant figure

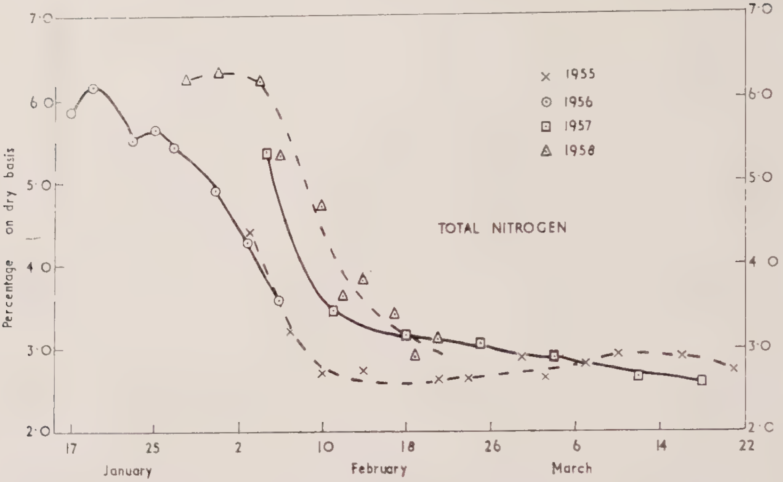


Fig. 6.—Variation in percentages of total nitrogen in the dry matter of developing cones for four seasons.

for 3 March, to 64.0 at the end of the season. In 1958, the range of variation is somewhat greater, this percentage falling from 70 at the beginning to 55 at the end of the season. This reduction in the proportion of nitrogen in the protein form is not unexpected in view of the change from tissues of probable high protoplasmic content to those of a more mature type, which as will be seen later contain an increased amount of structural material with a relatively high pentosan content.

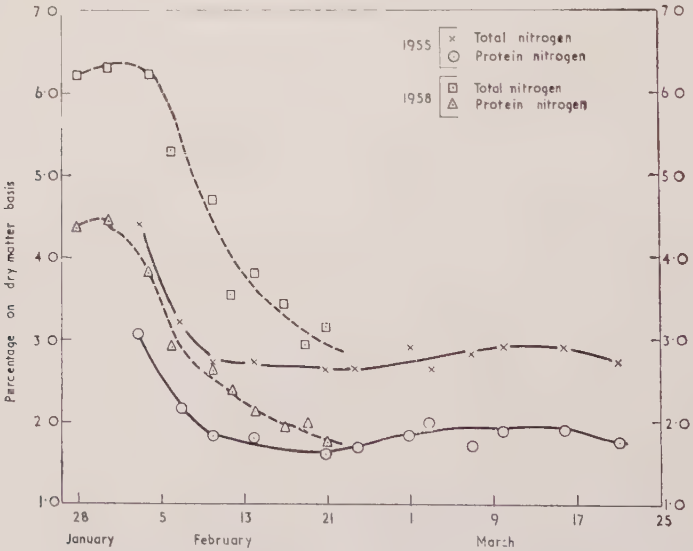


Fig. 7.—Variation in percentages of total and protein nitrogen in the dry matter of developing cones in the 1955 and 1958 seasons.

By combining the data of Figs. 1, 3, 4, 5, and 6, and expressing them graphically, Figs. 8 and 9 are obtained; these show the intakes of nutrients in milligrammes per cone at the various stages of development. Taking Fig. 8, dealing with the minute flowers and diminutive cones of the 1956 sampling period, it is seen that after the first two samplings intake of nutrients was appreciable, and proceeded at a rapidly increasing rate as the flowers came into burr and the cones developed. All the curves are essentially the same shape, but intake of soluble ash, potash, and nitrogen occurred to a much greater extent than of calcium, magnesium, and phosphorus. The point (25 January) at which the rates of intake increase rapidly corresponds to the stage where the flowers were in burr and some small cones were present: after that date there was progressive enlargement of the cones with rapid increase of dry matter content (Fig. 1), and of intake of minerals (Fig. 8). It is interesting to note that intake of potassium is doubled each 5 days, but that the rates of intake for the other constituents are slightly slower, the time required for them to be doubled being about 6 days for nitrogen, and 7 days for calcium, magnesium, and phosphorus. But even so the amounts per cone were only a few milligrammes or only a fraction of a milligramme, at the end of the sampling period.

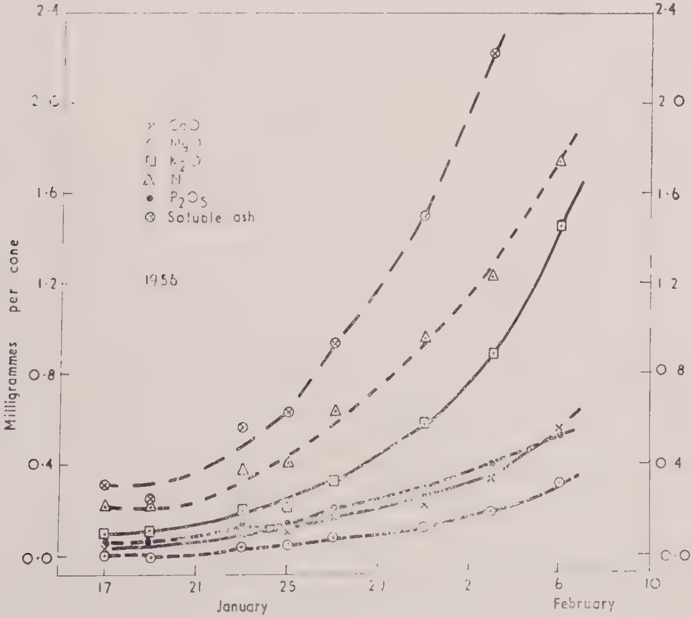


Fig. 8.—Accretion of soluble ash, calcium, magnesium, potassium, phosphorus, and nitrogen, in milligrammes per cone for very young cones, 1956 season.

The data of Fig. 8 are incorporated on the left of Fig. 9, but owing to the difference in scale the intakes appear to be very minute. It is clear however that the curves for the 1956 and 1957 data form continuous sequences for the respective constituents. With continued growth the soluble ash increased rapidly to reach a final value of nearly 24 mg per cone; intakes of nitrogen and potassium were large, amounting finally to 8.0 mg and 9.1 mg (as K_2O) respectively. Calcium, phosphorus, and magnesium intakes decreased in that order, being for the final sample, 4.1 mg (as CaO), 3.0 mg (as P_2O_5), and 1.7 mg (as MgO), respectively. It is noticeable too that towards the end of sampling, when the cones were over-mature for commercial purposes, intake of nitrogen, calcium, magnesium, and phosphorus had ceased, but that potassium and soluble ash were still being built into the cones.

Sugars Content and Accretion of Sugars

Data for the 1955 season are not given in full because the variations were similar to those found in each season. It may be of interest however to note that the maximum reducing sugars content was 8.73% obtained for material collected on 10 February, when the young hop cones were ½ inch to 1 inch long. Of these sugars 4.48% was glucose and 4.25% fructose. Sucrose content in this season rose to 2.29% on 14 February. After those dates, with the cones approaching full size, the sugars all decreased rapidly in concentration in the dry matter. The only other notable point respecting sugars in this season was that the maximum glucose content (4.74%) was found on 7 February, but the fructose maximum (4.40%) was obtained on 14 February, when maximum increase was present. However, on plotting the season's

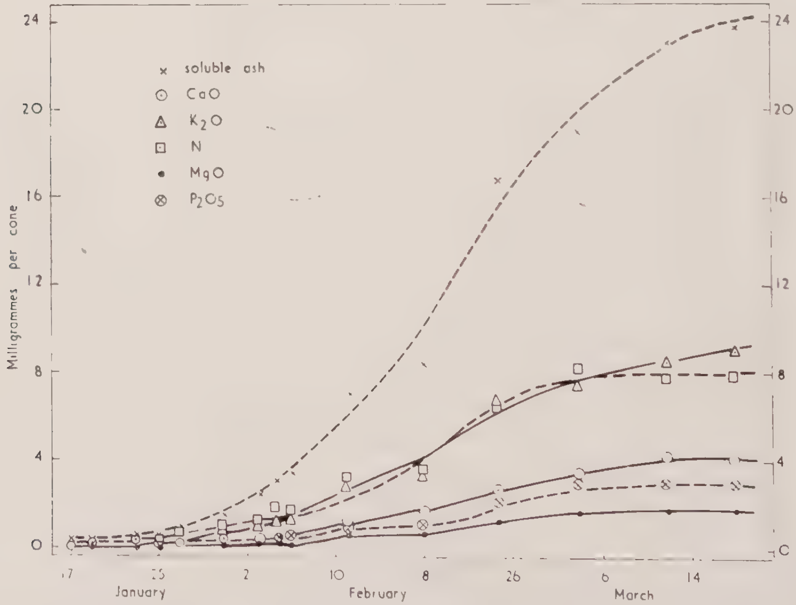


Fig. 9.—Accretion of soluble ash, calcium, magnesium, potassium, phosphorus, and nitrogen, in milligrammes per cone during development, 1956 and 1957 seasons.

data there is an indication that the glucose maximum may have been found on 9 February and the fructose one on 12 February, had samples been taken on those two days.

In Fig. 10, data are plotted for the glucose, fructose, and sucrose contents of the samples from the 1956 and 1957 seasons. In 1956 the total reducing sugars, composed essentially of glucose in the early samples, rose rapidly from 0.6% on 17 January, previous to burring, through 3.3% on 31 January, when the small cones were growing rapidly, and 4.5% for well-formed cones on 3 February to the high figure of 8.4% on 6 February, the final date of sampling. For this last sample glucose and fructose were present in equal amounts. Sucrose was low throughout, the highest figure being 0.7% for the final sample. Obviously in this season sampling stopped at, or just before, the time of maximum sugars content.

The curves for the reducing sugars in the 1957 samples are parallel to those of 1956, the displacement in position in Fig. 10 being probably a seasonal effect bringing about varying stages of growth between the 1956 and 1957 seasons at a given date. A very rapid rise in reducing sugars occurred after the first sample on 5 February; on this date total reducing sugars amounted to 3.5%, of which glucose comprised 2.4%. By 11 February, when some cones were fully formed, total reducing sugars had risen to 8.0%, of which 5.2% was glucose. In this season the maxima for glucose and fructose contents were found on this date. Thereafter sugar contents fell precipitously, the total reducing sugars

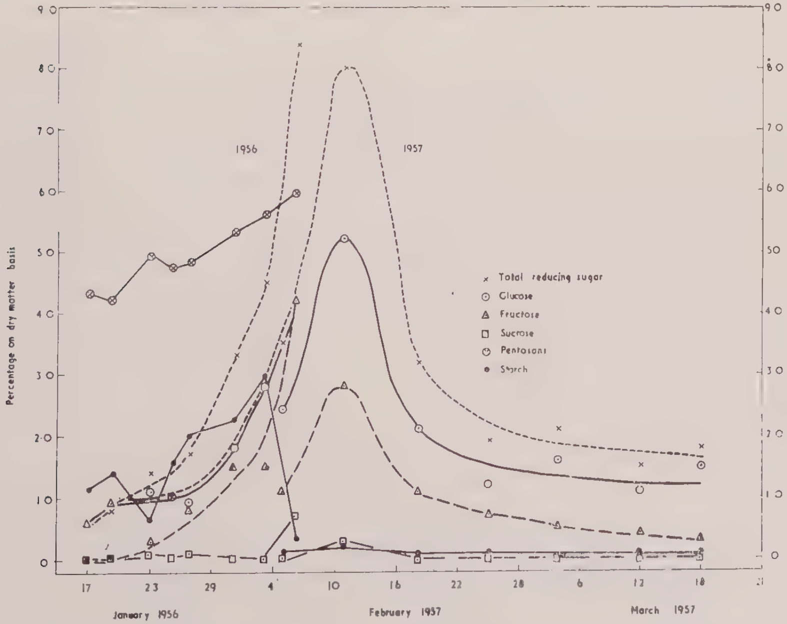


Fig. 10.—Variation in total reducing sugars, glucose, fructose, sucrose and starch in developing cones for the 1956 and 1957 seasons, and for pentosans in 1956.

being only 3.2% on 18 February, with glucose at 2.1%. At the end of the sampling period on 18 March total reducing sugars stood at 1.8%, of which 1.5% was glucose. Throughout this season fructose was consistently about one half, or less, of the amount of glucose. Sucrose in this season's samples was so low as to be undeterminable by the methods used, except that 0.3% was found for the sample of 11 February, the same date as that of maximum glucose and fructose content.

Further data, given in Table 1, refer to samples of the 1958 season, when 10 samples were taken over a 24-day period, which, it was estimated, would cover the period of rapid changes in sugars contents. As will be seen from the table this expectation was realised.

TABLE 1. SUGARS AND STARCH CONTENTS OF DEVELOPING CONES (% of D.M.)

Date of Sampling	Glucose	Fructose	Sucrose	Starch
28.1.58	2.01	0.88	1.45	0.68
31.1.58	1.08	0.41	0.61	0.82
4.2.58	1.21	0.74	0.48	0.81
6.2.58	2.03	1.95	1.09	0.04
10.2.58	3.42	3.93	2.08	0.36
12.2.58	4.11	6.23	1.67	0.42
14.2.58	3.62	6.57	1.16	0.12
17.2.58	2.35	2.97	1.03	0.06
19.2.58	2.55	3.20	0.91	0.08
21.2.58	1.63	2.19	0.43	0.10

The first sample of Table 1 consisted of flowers in full burr. By 31 January they had lost their furriness, in general, with some now in the form of small cones, 1/6 inch to ¼ inch in length. Sugars content was relatively high but decreased to a minimum in the sample of 4 February; at this time the cones were about ½ inch long. Thereafter reducing sugars content increased rapidly to the high maximum figure of 10.34% on 12 February; on this date seeds and traces of lupulin were present, these having been first noticed in the larger cones of the sample obtained on the 10th. There was a slight fall in sugar content on the 14th, with very rapid reduction after this date. Sampling stopped on 21 February with the sugar content still relatively high in these immature cones.

In other seasons the glucose content at any given stage of growth was usually equal to, or greater than, that of fructose, but, in 1958, glucose, except in the first four samples, was present in lesser amount than fructose. The maximum values for glucose and fructose in any given season are usually found in the same sample. What the factor may be which decides the relative proportions of glucose and fructose is quite unknown, but obviously there is scope for further work to try to determine it.

Sucrose contents in 1958 were relatively high and varied in sympathy with the reducing sugars in a more consistent manner than in 1956 and 1957, when sucrose contents were low throughout the sampling periods. In 1955, however, sucrose contents were comparable with those of 1958, the maximum figure in the former season being 2.29% on 14 February, just after the point of maximum reducing sugars content; at this time the cones were about 1½ inches long. The cause of the wide variation in sucrose content from season to season is not known.

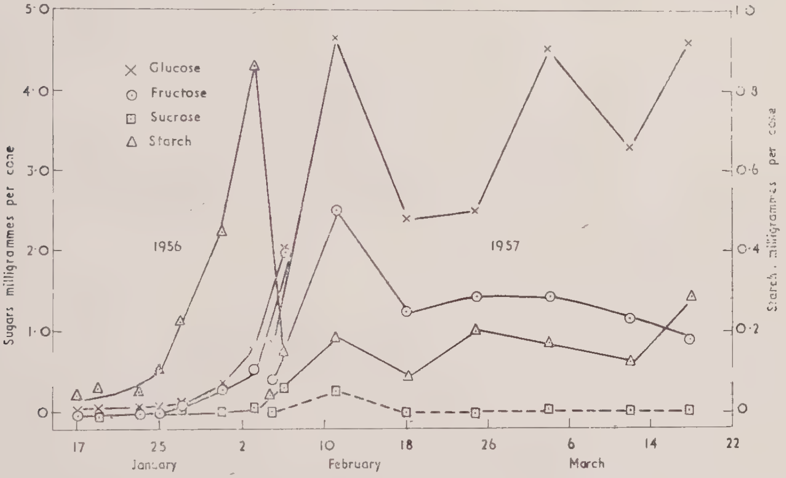


Fig. 11.—Accretion of glucose, fructose, sucrose, and starch, in milligrammes per cone for the 1956 and 1957 seasons.

The amounts of glucose, fructose, and sucrose, expressed as milligrammes per cone, in the young flowers and developing cones, are shown in Fig. 11 for the 1956 and 1957 seasons, and in Table 2 for the 1958 season.

TABLE 2. Sugars and Starch Contents (mg per cone) for Developing Cones of the 1958 Season

Date of Sampling	Glucose	Fructose	Sucrose	Starch
28.1.58	0.13	0.06	0.10	0.05
31.1.58	0.10	0.04	0.06	0.08
4.2.58	0.22	0.14	0.09	0.15
6.2.58	0.66	0.64	0.36	0.01
10.2.58	1.94	2.23	1.18	0.20
12.2.58	4.08	6.18	1.66	0.42
14.2.58	3.56	6.46	1.14	0.12
17.2.58	2.97	3.75	1.30	0.08
19.2.58	3.50	4.39	1.25	0.11
21.2.58	2.55	3.42	0.67	0.16

From Fig. 11 it is seen that in the young flowers and minute cones (1956 season) build-up of sugars is slow at first, but that after burring and the appearance of definite cones the sugars increase rapidly, this being especially marked for glucose and fructose. The data for 1957 continue smoothly with those of 1956. Maximum sugar content per cone was found on 11 February, by which time the cones were well-formed. All three sugars were maximal at the same time.

In 1958, maximum figures for glucose and sucrose, and a high figure for fructose, were found for the sample of 12 February, at which time seeds and lupulin had appeared in the well-grown cones. In 1957 and 1958, during periods which would cover rapid growth of the cones with increase in size of seeds and in amount of lupulin, the quantities of sugars decreased, but later increased again. All three sugars rose and fell together in the rapid-growth period, but did not necessarily vary in the same direction later in the season. Later on in this paper the variations in sugar content will be discussed in relation to the amounts of alpha resins and petroleum-ether-soluble substances present in the cones.

Content and Accretion of Starch

Data for the starch contents of the samples of the 1956 and 1957 seasons are given in Fig. 10 so as to present as complete a picture as possible on the one diagram of the carbohydrates in the growing cones. They are also given, on a larger scale, in Fig. 12, together with data for the 1955 and 1958 seasons.

Owing to shortage of material it was not possible to determine the starch content of the sample of 3 February 1955, but, judging by the form of the curve plotted from the data for this season, it would be expected to be possibly higher than the 1.45% found for the sample of 7 February. The very small flowers of the 1956 season averaged 1.25% just after mid January, but this value fell to 0.62% on the 23rd. This was followed by a rapid rise to 2.97% on 3 February, and by an extremely rapid fall to 0.31% on the 6th. Thus the rise from 0.62% to 2.97% and then back to 0.31% took place over a period of only 14 days. In 1958 a similar, although much less spectacular, rise and fall in starch content also occurred. By mid February in the three seasons, 1955, 1957, and 1958, starch content was in all cases very small, and as the cones approached commercial maturity it was only 0.05% or lower.

In 1956, as shown in Fig. 10, the starch content rose with increasing reducing-sugars content, the maximum starch figure of 2.97% corresponding to a reducing-sugars content of 4.5%: but while the reducing-sugars content increased to 8.45% the starch content fell back to 0.31% in three days. Because of cessation of sampling at this stage it is not possible to say what the further relationship of sugars and starch might have been.

In 1958, the highest starch contents were found for the first three samples of the season, a rapid fall from 0.81% to 0.04% being experienced at the time when reducing sugars were increasing rapidly. When the reducing sugars were at their maximum the starch content was 0.42%. Following this period it fell steadily to values of 0.1% or less, the sugars also decreasing in concentration at the same time (see Table 1).

Therefore in both seasons high starch content was correlated with the stage of initiation of rapid increase in reducing sugars contents, but very high sugar content was accompanied by low starch content.

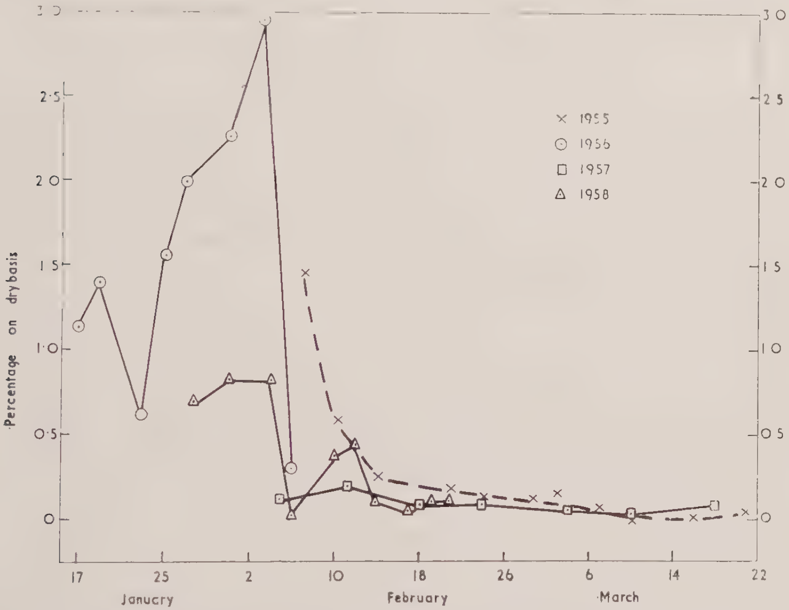


Fig. 12.—Variation in percentage of starch in developing cones for the seasons 1955 to 1958.

The 1956 and 1958 data, together with those of 1955, indicate that seasonal conditions may exert a great influence on the height to which the starch content of the growing flowers and cones may rise. On the other hand there may be here an influence of the time of the day, whether morning or afternoon, or conditions of sunlight and cloud, on the level of starch at the time of sampling. Obviously further work on this matter is necessary.

Accretion of starch for the minute flowers and young cones of the 1956 season, and of the young cones through to mature cones of the 1957 season, are included in Fig. 11, where the data are expressed in milligrammes per cone. The scale for starch content is five times greater than that for the sugars, which has, at first sight, the effect of exaggerating the amount of starch in the young cones especially. However the amounts of starch are of the same order, in the very young flowers and cones, as the glucose and fructose contents. At the high peak, on 3 February, the amount of starch is only 0.86 mg per cone. Up to this time the amount of starch per cone was doubled in three days. Over the sampling period of 1957 the starch figures tend to follow the variations in the sugars. Accretion of starch in 1958 followed a somewhat similar pattern, but at a much lower level. The maximum amount per cone in this season was only 0.42 mg, on 12 February, when the amounts of glucose, fructose, and sucrose were maximal, and when lupulin was appearing in the cones (see Table 2). It should not be thought however that starch and sugars are built into the cones and remain there as such; this cannot be, because growth and development are dynamic processes. The data merely refer to the state of the cones at time of sampling.

Pentosan Content and Accretion of Pentosans

The make-up of the developing hop cone in respect of free and condensed pentose sugars is not known, only the mature cones having been examined by previous workers. On the present occasion water-soluble and water insoluble compounds have not been reported separately, the data being given as total "pentosan." There is some evidence that water-soluble compounds, which have been estimated as "pentosan", are present even in the flowers and very young cones, and that methyl pentoses are also present; but the investigation of these has not been sufficiently developed to warrant inclusion of the data here.

In Fig. 13 the percentages of pentosans for the developing flowers and very small cones of the period 17 January to 6 February 1958, and for the period from just past maximum burr (5 February) to over-maturity (21 March) for the 1955 season are set out. It is clear from

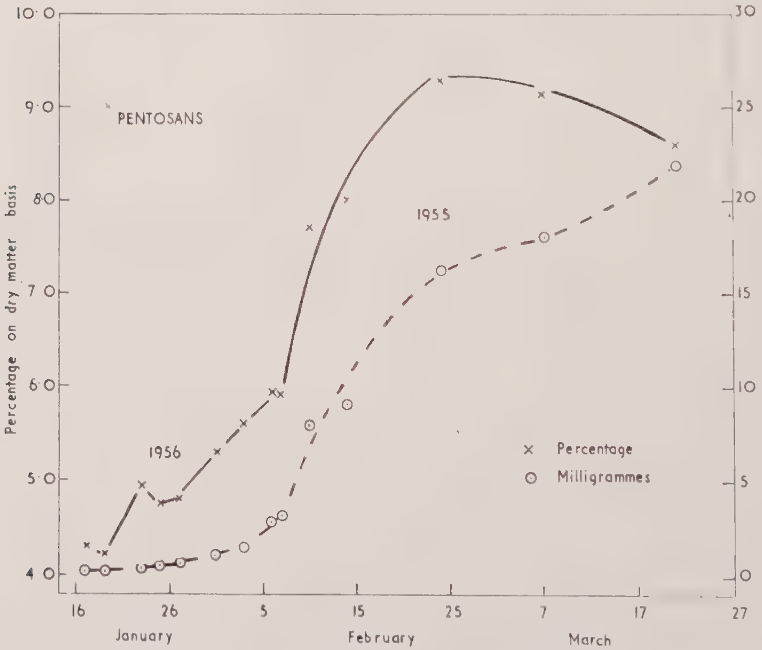


Fig. 13.—Percentage in the dry matter, and accretion in milligrammes per cone, of pentosans in developing cones of the 1956 and 1957 seasons.

this figure that the percentages of pentosans for the very young material, rising from 4.2% to 5.9%, join up very satisfactorily with the figures for the 1955 samples. In the latter, on 7 February the pentosans content was 5.9%. Pentosans continued to increase to the maximum figure of 9.3% on 24 February, just about the time that harvesting of the crop began. With increasing maturity the pentosan content of the dry matter decreased somewhat to 8.6%, for over-mature cones, on 21 March. It will be noted that there is a distinct maximum, at 4.95%, for the sample of 23 January 1956; this was accompanied by decreases in the percentages of nitrogen and starch. At present these facts can merely be recorded, without offering any interpretation of them.

The accretion of pentosans, in milligrammes per cone, is also given in Fig. 13. For the flowers and minute cones the amounts vary from 0.16 mg in the first two samples to 2.9 mg in the last one on 6 February 1956. Over the sampling period, the amount per cone is doubled every 4 to 5 days in the early samples, and every 3 to 4 days later on. The curve for the 1956 samples joins neatly with that of the 1955 samples. After the first week in February 1955 accretion of pentosans was rapid, rising from 3.1 mg on 7 February through 9.1 mg on the 10th to 16.3 mg on the 24th, and finally to 21.9 mg in the last sample on 21 March. Over the period when the pentosans were increasing rapidly the amount per cone was doubled every 5 days; later on accretion was somewhat less rapid. It will be noted that the time required to double the content of pentosans is very similar to that required to double the amounts of other constituents such as dry matter, starch, nitrogen, and minerals.

Resins

Data for the constituents soluble in petroleum ether (b.p. 40–60°c) are available for the sample material of the 1956, 1957, and 1958 seasons. In Fig. 14 the alpha-resins (precipitable by lead) and the total solids, soluble in petroleum ether, are given for the 1956 and 1957 seasons. For the former season the first sample analysed was obtained on 25 January; shortage of material did not permit determinations of the resins in the earliest samples. Alpha-resin content, which is the more important constituent from a quality viewpoint, rose from 0.4% on 25 January to a slight maximum of 0.8% on the 31st, to decrease to the low figure of 0.3% for the last sample (6 February). Total soluble solids increased steadily from 1.3% to 2.2% at the end of January, and then rose rapidly to 4.3% between 3 and 6 February. It would appear that by 6 February 1956 formation of resins was well under way and, if sampling had been continued further, this would have continued in a way similar to that of the 1957 samples, which show a rapid increase after 11 February, when many well-formed cones were present. In this latter series the maximum alpha-resin and total soluble solids contents were respectively 9.52% and 17.92%. The fall in value of both constituents for the last sample on 18 March is doubtless due to over-maturity of the cones.

Corresponding data for alpha-resins and total soluble solids for the 1958 season are given in Table 3.

TABLE 3. PERCENTAGE OF ALPHA-RESINS AND TOTAL SOLUBLE SOLIDS IN THE DRY MATTER, AND WEIGHTS OF THESE CONSTITUENTS IN MILLIGRAMMES PER CONE

Date of Sampling	Percentage in Dry Matter		Milligrammes per Cone	
	Alpha-resins	Total Soluble Solids	Alpha-resins	Total Soluble Solids
28.1.58	0.08	0.81	0.005	0.05
31.1.58	0.12	0.95	0.011	0.09
4.2.58	0.03	0.85	0.006	0.16
6.2.58	0.04	2.11	0.013	0.69
10.2.58	0.08	3.90	0.045	2.22
12.2.58	0.38	5.40	0.38	5.36
14.2.58	0.09	5.15	0.09	5.06
17.2.58	0.07	7.65	0.09	9.66
19.2.58	0.90	5.45	1.23	7.47
21.2.58	3.01	11.57	4.70	18.07

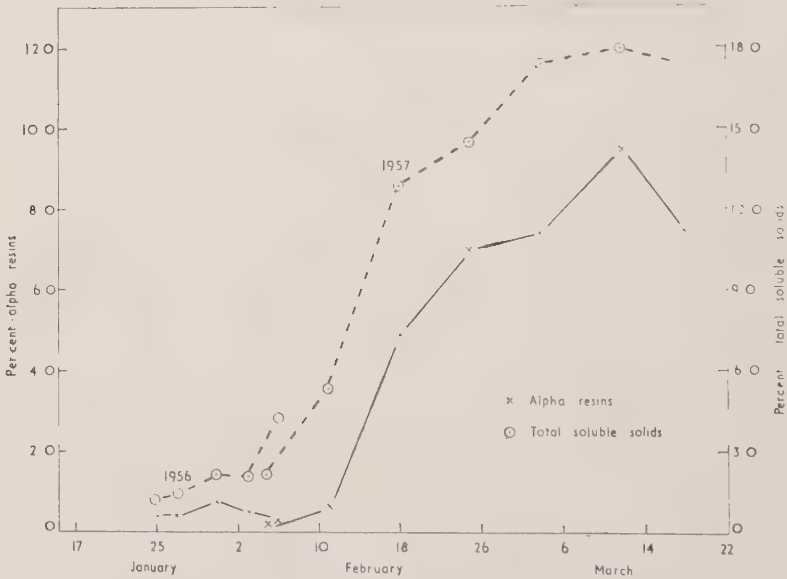


Fig. 14.—Percentage of petroleum-ether-soluble solids and alpha-resins in developing cones of the 1956 and 1957 seasons.

In 1958 the percentages of alpha-resins were much lower than in 1957, except for the sample of 12 February, for which the figure of 0.38% is comparable with that of 0.59% for 11 February 1957. In view of the lack of samples between 11 and 18 February 1957 it is not possible to say whether decreases in alpha-resin content would have been found for that period in the same way that the low values occurred on 14 and 17 February 1958. It is not to be expected however that the cones should give the same analytical figures for samples taken on a specified date in each season. The highest figure, 3.01%, for the 1958 season was obtained with the last sample on 21 February. This figure may be compared with those of 4.92% on 18 February, and of 6.97% on 25 February 1957.

For total soluble solids much the same state of affairs for the analytical data of the 1957 and 1958 seasons is found, the samples of the latter season being lower in soluble solids than those of the former. In 1958 the total solids rose from 2.11% on 6 February to 11.57% on 21 February; these may be compared with 2.10% on 5 February and 14.50% on 25 February of the 1957 season. In the 1958 season seed and resin (lupulin) development were noticeable in the sample of 10 February, and the typical hop aroma was first obvious a week later; over this period alpha-resin content was still very low but the total solids had begun to increase appreciably. The lower resin contents for a given date of the 1958 season are probably due to the poorer seasonal conditions as compared with the previous season, a fact generally conceded by growers: moreover the low figures are also probably related to the large amount of seed in the cones in 1958.

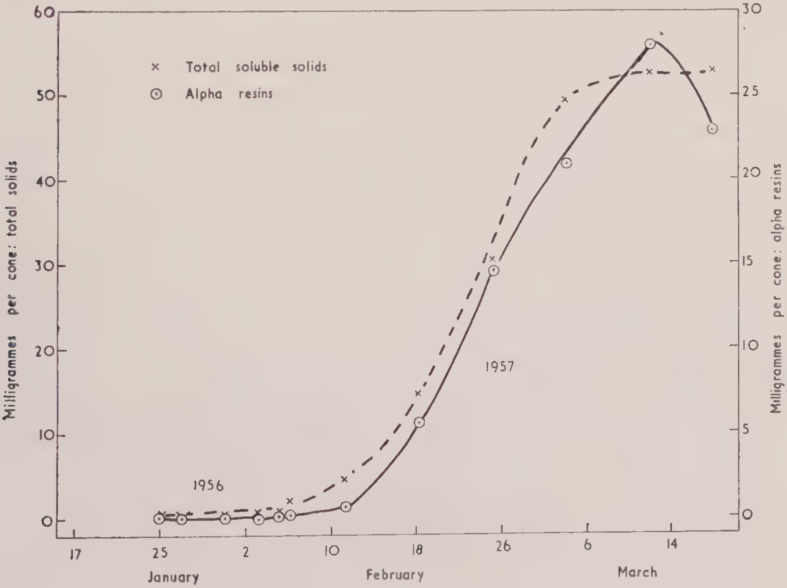


Fig. 15.—Accretion of soluble solids and alpha-resins during growth of cones, 1956 and 1957 seasons.

Accretions of both alpha-resins and total soluble solids have been calculated for three seasons, those for 1956 and 1957 being plotted in Fig. 15, and tabulated for 1958 in Table 3. In Fig. 15 the amounts of alpha-resins per cone increased from 0.03 mg on 25 January 1956 to 1.5 mg at the end of the sampling period on 6 February. In the following season the alpha-resins increased rapidly from 0.07 mg on 5 February, through 0.53 mg on 11 and 5.60 mg on 18, to the maximum of 28.1 mg on 12 March, followed by a decrease to 22.9 mg in the overmature sample of 18 March. Table 3 shows that the amounts of alpha-resins in the young cones of the 1958 season were minute, and that by 21 February there was only approximately half the amount that was present at that date in the previous year. With approaching maturity of the cones the alpha-resins were increasing rapidly, the final level to which they might have increased not being determined because of cessation of sampling on 21 February.

Total soluble solids are shown in Fig. 15 to increase rapidly from about mid February onwards to give a sigmoid curve, which does not show a loss of solids in the last sample as was found for the alpha-resins. In the very young flowers and cones of the 1956 season the soluble solids increased from 0.09 mg on 25 January, through 0.60 mg on 3 February to the relatively high figure of 2.09 mg on 6 February. The data for 1957 follow on to give a smooth curve with those for 1956; in the former season there was 4.81 mg on 11 February, which, as shown in Fig. 15, rapidly increased through the figure of 14.7 mg on 18 February to 49.4 mg on 4 March and to the practically constant levels of 52.9 mg and 53.1 mg for the last two samples. A similar type of variation is shown by the 1958 data of Table 3, there being a steady build-up of soluble solids with growth of the cones, especially after the development of the typical hop aroma about 17 February.

In previous publications (Askew, Monk, and Hodgson, 1954; Askew and Monk, 1955) attention has been drawn to the inverse linear relationship between sugars and resins contents. This same relationship has been found to hold for the last five samples of the 1957 season, that is, for cones approaching and at maturity.

DISCUSSION

Seasonal conditions can be expected to affect the chemical composition of any crop plant so that, while the same type of variation of concentrations of constituents may occur each year, the quantitative differences between one season and another may be appreciable. This is what has been shown by the data presented above for the developing hop cone. It is true also that the data for a sample taken at any particular instant can refer only to the material at that instant, without there being often any measure of the dynamics of those constituents. Thus, intake of minerals may be considered to be a one-way process, because the mineral constituents may not be so mobile that they pass first into the cones and then out again; it would seem probable that once they have entered the cone they will stay there. This question could probably be answered by the use of radioactive isotopes of the elements concerned. Nitrogen may be on a slightly different basis: nevertheless it would seem that redistribution of nitrogen from the cone would not be great. There is much yet to be discovered concerning the part nitrogenous compounds play in the growth processes. But when the organic constituents are considered the problem becomes somewhat more complex. Take for example the carbohydrates. From the data presented, the content of starch varied greatly throughout the season, but the level has been very different from one season to another for comparable material. Obviously further work is required to try to unravel the influences of the several possible conditions of growth and season on the starch content. Except in the early part of the season, build-up of starch would appear to be small. May not this be due to the lability of the starch, it being further metabolised, and also because of the changes in structure of the flower and cone? On the other hand pentosan content has been appreciable in all samples examined, and has progressively increased as the cone reached maturity, on both a percentage and a quantity basis. It would appear therefore that once pentosans have been formed they may not be a ready source of metabolisable material.

Reducing sugars contents have been shown to vary greatly, with relatively high concentrations in young cones, and with increase in the weights of sugars leading in some cases to maximal amounts, usually early in the growth of the cones. This applies to all three sugars, glucose, fructose, and sucrose. With development of resins, the sugar content, on both a percentage and a quantity basis, is reduced, doubtless owing to these (and other) constituents being used in the production of resins. Further work is required to determine whether any other sugars are present, and how they may vary in amount during growth of the cone. Moreover, further information is required on the effect of meteorological conditions on the level of sugars in the cones.

Several other possible constituents such as organic acids, amino-acids, pectins, tannins, and oils, also should be investigated in order that as complete a chemical picture as possible of the manner of growth of the cones may be obtained.

The chemical reactions taking place during the development period of the cone are complex, but it is clear from the data presented in this paper that constant patterns of change in the concentrations of various constituents take place in constant sequence. Also, increases in some constituents accompany decreases in others. Although the present data do not permit conclusions to be made as to the precise part each of the constituents plays in the metabolic processes, yet the data provide much information that is of interest. To obtain data on the parts played by the several constituents would require the use of tracer techniques.

REFERENCES

ASKEW, H. O.; MONK, R. J.; HODGSON, JOYCE 1954: *N.Z. J. Sci. Tech. B36:* 227.

ASKEW, H. O.; MONK, R. J. 1955: *J. Inst. Brew. 61:* 32.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (A.O.A.C.) 1945: "Methods of Analysis," 6th ed., p. 412. Association of Official Agricultural Chemists, Washington, D.C.

FORD, J. S.; TAIT, A. 1932: *J. Inst. Brew. n.s. 29:* 351.

PUCHER, G. W.; LEAVENWORTH, C. S.; VICKERY, H. B. 1948: *Anal. Chem. 20:* 851.

SOMOGYI, M. 1945: *J. biol. Chem. 160:* 161.

CONTROL OF *Pieris rapae* BY GRANULOSIS VIRUSES

By J. M. KELSEY, Entomology Division, Department of Scientific and Industrial Research, Ashburton

(*Received for publication, 12 May 1958*)

Summary

Laboratory tests with two strains of granulosis virus—one from New Zealand and the other from England—showed that 100% control of *Pieris rapae* L. larvae was achieved in 9 and 11 days respectively. Results of sampling in 61 different areas in home gardens or on farms showed that deaths of caterpillars due to virus were not common up to the end of January, but that by the end of the first week in February the mortality rose to nearly 20% of white-butterfly larvae; then to approximately 57% by 21 February; to 75% during the last week in February, 77% in March, and then fell slightly to 70% during April. During the latter two months and late February, however, there were nine cases where complete control of larvae was recorded, and a further ten cases where control percentages were very high.

INTRODUCTION

The New Zealand virus has been tentatively identified by Steinhaus (in litt.) as *Bergoldia virulenta* Tanada and is the same one as recorded by the writer in a previous paper (Kelsey 1957). The English virus strain was forwarded by Dr. Kenneth M. Smith who prepared it from *Pieris brassicae* L. larvae that died from 21-23 November 1955; this virus has been kept at Ashburton at ordinary room temperatures, and was nearly 16 months old at time of setting up tests. The New Zealand strain was freshly prepared from larvae that died in early March 1957.

TECHNIQUE

Tests commenced on 5 March 1957 and were carried out in a laboratory at normal temperatures using 5 larvae per 6-in. petri dish with 10 replicates for control and 15 for treatments; i.e. totals of 50 larvae for control and 75 for treatments. Food consisted of freshly picked rape leaves each day, and dishes were washed daily in hot running water after transfer of larvae to clean dishes. The virus was applied by dipping healthy rape leaves in an aqueous preparation of the virus concentrate. As this preparation by itself would not adhere satisfactorily to the leaves, a quantity of liquid detergent was added. The proportions in the dip were 3.5 c.c. of virus concentrate (50 macerated infected larvae in 10 c.c. of water), 3 c.c. of liquid detergent and 300 c.c. of water. The controls were dipped in water to which the liquid detergent was added at the rate of 3 c.c. to 30 c.c. of water. It was found that the wetting agent, at the concentration used, killed healthy larvae that were placed on leaves that were still wet after dipping in these solutions, and for this reason caterpillars were not placed in dishes until solutions had dried on leaves. Under these conditions the detergent did not damage caterpillars. Both virus concentrates were heavily contaminated by bacteria which usually appeared soon after death of virus-killed larvae,

N.Z. J. agric. Res. 1: 778–782

but were not present during life of virus-infected caterpillars. Test larvae were collected from one source, and only 3rd, 4th, and 5th instars were used in approximately even numbers in treatments and containers. Larvae were permitted to feed on treated leaves for only 24 hours, after which their food was uncontaminated leaves. Daily records were kept. Results are given in Table 1.

TABLE 1. CONDITION OF LARVAE

Date March	Days after Feeding on Virus-treated Leaves	Control		N.Z. Virus		English Virus	
		Pupated	Virus	Healthy	Virus	Healthy	Virus
6	1	0	0	75	0	75	0
7	2	0	0	75	0	75	0
8	3	1	0	69	6	73	2
9	4	4	0	66	3	70	3
10	5	3	0	65	1	69	1
11	6	9	0	61	4	68	1
12	7	4	0	45	16	50	18
13	8	12	0	29	16	27	23
14	9	11	2	0	29	8	19
15	10	2	1	—	—	2	6
16	11	1	0	—	—	0	2
Totals killed by virus:			3		75		75

NOTES: Pupated larvae were probably virus-free as indicated by the fact that they produced normal butterflies from 21 March onwards.
The liquid detergent used was Teepol.

DISCUSSION

Results in the table show that, under the conditions of the experiment, 100% control of *Pieris rapae* larvae was achieved by both the New Zealand and English strains of virus, even though the latter originated in *Pieris brassicae*; this shows that this virus is not specific in its host. The test also showed that presence of virus prevented pupation of larvae, even when virus was present for only 3 or 4 days, at which stage pupation had commenced in controls of the same instars. First deaths occurred with both viruses on the third day after feeding on contaminated leaves, and peak mortalities after 8 and 9 days for English and New Zealand strains respectively. Though pupation of larvae was prevented by the virus, feeding by the larvae that had just entered the final instar was extended over a longer period than was the case in controls of approximately the same age, with the result that more damage to leaves occurred. Presence of virus did not prevent ecdysis between instars. The 3 positive virus deaths in controls were due probably to some of the food material used towards the end of the experiment being virus-contaminated.

Smith, in a letter accompanying the virus concentrate from *P. brassicae* larvae, reported that it killed the latter within about 4 days, and while it also killed *P. rapae,* he suspected that the *brassicae* strain merely triggered off a granulosis virus already latent in *rapae* larvae. The present test indicated that from infection to death of larvae there were no real symptomatic differences between the two strains of virus, and electron micrographs taken by Dominion Physical Laboratory, Wellington, indicate that the two viruses are of the same general type. (Fig. 1.)

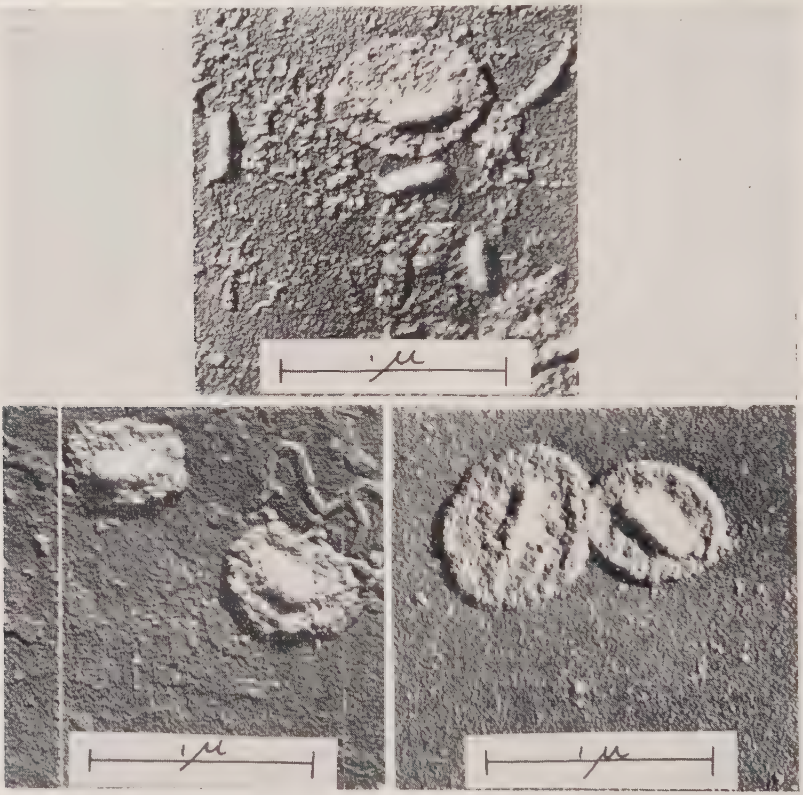


Fig. 1.—Top: Granulosis virus from *P. brassicae* in England. Lower left: Granulosis virus from New Zealand *P. rapae* larvae artificially infected with *P. brassicae* virus. Lower right: Naturally occurring *Bergoldia virulenta* virus from *P. rapae* larvae in New Zealand. All × 29,000. These electron micrographs do not show the actual virus particles (in this case, cigarette-shaped rods). In the top micrograph, some inner capsules which are known to contain the virus rods (Tanada 1953) are shown free of the outer capsules. In the lower micrographs, the inner capsules are clearly observable inside the outer capsules. The granular nature of both inner and outer capsules is shown in all three micrographs. All virus samples were treated for one minute with 2% sodium carbonate solution.

Field Test

On 10 March a rape plot measuring 28 x 6 yards was sprayed with 20 c.c. of New Zealand virus concentrate in 2 gal. water containing

8 c.c. wetting agent. This area was already showing slight signs of virus infestation at that date (21 out of 2,290 larvae were virus-affected). By 21 March all larvae seen (3,881) were killed by virus, whereas on an adjacent plot of the same size sprayed with the wetting agent and water only there were 311 apparently healthy and 2,047 virus-killed larvae. In this test it was noted that when virus was present at egg-hatching, larvae were killed by the virus by the time they reached late 2nd instars, and very little damage was done to the crop by caterpillars, but if virus was not present by the time the third instar was reached, and infection then occurred, there was a feeding period of up to ten days during which extensive damage occurred. This would indicate that if a crop were sprayed with a virus culture when first-generation butterfly eggs were hatching, it may be possible to prevent subsequent damage to that crop.

TABLE 2. CONDITION OF LARVAE IN NATURAL FIELD INFECTIONS OF VIRUS

Early February

Healthy	Virus
117	24
43	9
147	5
19	11
62	47
8	2
396	98

Mid February

Healthy	Virus
86	63
204	337
14	2
21	11
156	208
315	477
81	47
877	1145

Late February

Healthy	Virus
95	74
111	59
166	184
9	14
49	30
180	405
78	33
88	197
14	93
0	230
311	2047
1101	3366

March

Healthy	Virus
539	799
420	69
691	842
151	89
204	90
56	41
11	1
57	803
7	663
30	5
5	9
23	8
11	61
82	104
37	15
109	101
58	19
7	231
0	49
108	344
19	177
5	29
0	11
3	111
11	80
5	237
0	35
0	9
0	37
19	66
4	74
0	27
0	3881
2672	9117

Early April

Healthy	Virus
0	39
151	403
33	49
208	451
392	942

NATURAL FIELD INFECTIONS

Table 2 gives results of inspections of crops throughout Canterbury from early February to early April 1957. Some cases were home gardens but most were farm crops.

These observations indicated that virus probably appears on crops during January and February by action of rain splashing the virus from the ground on to lower leaves, or by the latter actually touching ground that had previously been in a cruciferous crop attacked by virus-infected caterpillars. At this early stage in natural virus infection it was found that larvae feeding on central leaves well away from the ground were rarely infected by virus. During late February and March, however, the damage caused by high larval populations resulted in caterpillars moving more or less haphazardly over plants in search of food and it is considered probable that larvae that had picked up virus from lower leaves carried it to top leaves. When caterpillars died and liberated the virus, the latter was splashed by rain over most of the plant, with the result that all larvae of subsequent generations were likely to become infected by virus. Another reason for a higher infection rate in later *Pieris* generations is that, just prior to death, virus-infected larvae usually moved to near the tops of leaves or plants, which resulted in all parts of the plant below these points becoming contaminated with virus.

ACKNOWLEDGEMENT

The writer is indebted to the Dominion Physical Laboratory, Department of Scientific and Industrial Research, for the electron micrographs.

REFERENCES

KELSEY, J. M. 1957: Virus Sprays for Control of *Pieris rapae* L. *N.Z. J. Sci. Tech. A38:* 644-6.

TANADA, Y. 1953: Description and Characteristics of a Granulosis Virus of the Imported Cabbageworm. *Proc. Hawaii ent. Soc. 15 (1):* 235-60.

THE LEG AND THE LOIN AS INDICES OF THE COMPOSITION OF NEW ZEALAND LAMB AND MUTTON CARCASSES

By R. A. Barton and A. H. Kirton, Massey Agricultural College, Palmerston North

(*Received for publication, 16 June 1958*)

Summary

The use of the leg and the loin as sample joints for estimating the carcass composition in terms of fat, muscle, and bone of 120 New Zealand lamb and mutton carcasses is discussed. In all cases the correlations between the dissectible components of these two joints and the same components of the whole carcass were highly significant. The correlation coefficients show that the combination of a leg and loin are better indices of carcass composition than either joint alone. Regression equations are presented for estimating carcass composition from the weights of the components of the leg and the loin. The use of the neck, thorax, shoulder, and pelvis as sample joints is also considered.

Introduction

The physical and/or chemical analyses of sample joints of carcasses are well established techniques for evaluating treatment effects on the various components of the carcass. In sheep studies, the leg and the loin singly or in combination have been most frequently examined and the dissectible components (fat, muscle, and bone) from these joints have been related to the same components in the whole carcass. Palsson (1939) for instance, found with 11 wether lambs and 5 wether hoggets of different breeding that in all cases the total weight of any dissectible carcass component could be accurately estimated, by using an appropriate regression equation, from the weight of the same component of the leg or the loin. More precise estimation, however, was achieved if one leg and the loin were used in combination rather than singly.

In the present paper these and other relationships have been considered for a relatively large number of New Zealand lamb and mutton carcasses.

Methods

Data from dissection work carried out by the Sheep Husbandry Department, Massey Agricultural College, were available for statistical analyses (c.f. Clarke and McMeekan, 1952). The 50 ewe and wether mutton carcasses* and 70 lamb carcasses studied had been selected from cold storage at a local meat works. The carcasses had been "telescoped" to reduce bulk in order to meet wartime shipping requirements. They were of unknown history but they covered the normal range in carcass weight and grade (Barton, 1947). Jointing of the carcasses into neck, thorax, shoulders, legs, loin (6 or 7 vertebrae), and pelvis, conformed to the anatomical regions as described by Palsson

---

* Carcasses in commercial practice are headless and the bones of the extremities including the metacarpals and metatarsals are removed. Perirenal fat is intact.

(1939). The joints were dissected into fatty tissue, muscular tissue, bone, and tendon plus "waste", again following essentially the method of Palsson (1939). One leg and one shoulder only were dissected for each carcass and the weights of the dissected tissues were doubled and added to the weights of the tissues from the other anatomical regions to give the total carcass components. The undissected weights of both legs and also for both shoulders from each carcass were almost identical so that errors arising from the dissection of one leg and one shoulder would be small. Perirenal-fat weight was excluded from the total weight of the fatty tissues of the carcass.

Details of the material analysed statistically are given in Table 1. The proportion of the different joints to the total weight of the carcass is shown in Table 2.

TABLE 1. MEAN COLD CARCASS WEIGHTS (g) AND WEIGHTS OF CARCASS DISSECTIBLE COMPONENTS (g) OF MUTTON AND LAMB

Carcasses	Item	Mean (g)	Range (g)
25 ewes[1] (Grade: Prime)	Carcass weight	27526	17191 – 36650
	Carcass dissectible fat	9748	4651 – 18568
	Carcass dissectible muscle	13444	9136 – 17113
	Carcass dissectible bone	2773	2095 – 3289
25 wethers[1] (Grades: Prime and Seconds)	Carcass weight	28012	17577 – 36060
	Carcass dissectible fat	9466	4232 – 16359
	Carcass dissectible muscle	13921	9599 – 17359
	Carcass dissectible bone	3060	2434 – 3620
33 wether lambs[2] (Grade: Prime Down Cross)	Carcass weight	17311	11748 – 22498
	Carcass dissectible fat	4698	2060 – 6243
	Carcass dissectible muscle	9177	6764 – 11804
	Carcass dissectible bone	2129	1617 – 2725
11 wether lambs[1] (Grades: Prime Crossbred and Seconds)	Carcass weight	18449	14515 – 21817
	Carcass dissectible fat	5735	4253 – 7338
	Carcass dissectible muscle	9345	7297 – 10892
	Carcass dissectible bone	2010	1457 – 2336
26 wether lambs[2] (Grades: Prime Down Cross, Prime Crossbred and Seconds)	Carcass weight	17166	12927 – 22997
	Carcass dissectible fat	5409	3447 – 8862
	Carcass dissectible muscle	8532	6627 – 11697
	Carcass dissectible bone	1811	1377 – 2210

[1] Breeding unknown but likely to be mainly Romney.
[2] Breeding unknown but likely to be mainly Romney-Southdown cross.

RESULTS

Leg and Loin

Fatty Tissue. The data in Table 3 show that in all cases the weight of the fatty tissue of the leg and the loin (singly and in combination) was highly significantly correlated with the total weight of the fatty tissue in the carcass. From the regression equations and the standard errors of estimate shown in Table 3 it can be seen that these two joints in combination give a more accurate estimate of total fat in the carcass

TABLE 2. JOINTS EXPRESSED AS A PERCENTAGE OF CARCASS WEIGHT

Carcasses	Region						
	Neck	Thorax	Shoulder (One only)	Loin	Leg (One only)	Pelvis	Leg + Loin
25 ewes	8.9	26.3	9.3	12.8	9.4	12.1	22.2
25 wethers	9.0	25.9	9.2	12.9	10.3	10.8	23.2
33 wether lambs	9.0	24.6	9.4	11.6	11.2	10.9	22.8
11 wether lambs	8.7	25.2	8.9	11.9	10.9	11.5	22.8
26 ewe lambs	8.6	24.7	9.1	12.3	10.7	11.4	23.0

Kidney-fat weight and jointing losses were not included in computing the percentages; hence the above data with shoulder and leg percentages doubled do not sum to 100.0.

than either joint alone. Of the two joints, however, the loin is the more accurate indicator of total carcass fat. When the standard error of fatty tissue weight for the leg plus loin was expressed as a percentage of the mean weight of carcass fatty tissue for each series, the error in fatty tissue prediction ranged from 5.7% to 8.5%.

Multiple regression was also used to predict carcass fatty tissue from the fatty tissue of the leg and the loin, using data derived from the series of 33 wether lambs. The multiple regression equation: total fatty tissue $= 5.558X_1 + 3.518X_2 + 502$ (where $X_1 =$ fatty tissue in leg (g);

TABLE 3. CORRELATIONS BETWEEN THE FATTY TISSUE OF THE LEG (g) AND THE LOIN (g) AND THE FATTY TISSUE OF THE CARCASS (g), AND REGRESSION EQUATIONS FOR PREDICTING THE WEIGHT OF CARCASS FATTY TISSUE FROM THE FATTY TISSUE OF THESE JOINTS

Dependent variate (weight of carcass fatty tissue in g) = Y

Carcasses	Independent variate = X	r	Regression equation	$s_{y.x}$ (g)
25 ewes	Fatty tissue of leg + loin	0.984	$Y = 4.178X + 955$	656
	Fatty tissue of loin	0.974	$Y = 5.099X + 1740$	807
	Fatty tissue of leg	0.945	$Y = 19.58X - 712$	1176
25 wethers	Fatty tissue of leg + loin	0.984	$Y = 3.646X + 1616$	616
	Fatty tissue of loin	0.982	$Y = 4.515X + 2445$	656
	Fatty tissue of leg	0.932	$Y = 16.67X - 503$	1256
33 wether lambs	Fatty tissue of leg + loin	0.978	$Y = 3.974X + 760$	327
	Fatty tissue of loin	0.966	$Y = 5.020X + 1470$	407
	Fatty tissue of leg	0.922	$Y = 15.50X - 695$	610
11 wether lambs	Fatty tissue of leg + loin	0.908	$Y = 3.865X + 1032$	488
	Fatty tissue of loin	0.853	$Y = 4.576X + 2089$	608
	Fatty tissue of leg	0.765	$Y = 10.79X + 1200$	749
26 ewe lambs	Fatty tissue of leg + loin	0.980	$Y = 4.048X + 604$	306
	Fatty tissue of loin	0.963	$Y = 4.935X + 1407$	415
	Fatty tissue of leg	0.886	$Y = 15.87X - 558$	710

All correlations significant at the 1% level.

X_2 fatty tissue in loin (g)) had a standard error of 324 g which was almost the same as the standard error of the simple regression equation (327 g) based on the combined weight of fatty tissue from these two joints.

Muscular Tissue. All the correlations shown in Table 4 between the weight of muscular tissue in the leg and in the loin (singly and in combination) and the total weight of muscular tissue in the same series of carcasses were highly significant. From the standard errors of the regression equations presented in the same table it is clear that the combined muscle weight of the leg plus loin gives a more accurate estimate of total carcass muscular tissue than either joint alone. The weight of the leg muscular tissue gives a better estimate of the whole carcass muscular tissue than does the loin. When the standard error of muscular tissue weight for the leg plus loin was expressed as a percentage of the mean weight of carcass muscular tissue for each series, the error in muscular tissue prediction ranged from 2.5% to 4.0%.

A multiple regression based on the weight of muscular tissue in the leg and the loin gave no greater accuracy of prediction of total carcass muscular tissue than did the simple regression presented in Table 4 for the series of 33 wether lambs.

Bone. The correlations and regression equations between the weight of bone in the leg and in the loin (singly and in combination) and the total weight of bony tissue in the carcass are presented in Table 5. All these correlations are highly significant. The standard

TABLE 4. CORRELATIONS BETWEEN THE MUSCULAR TISSUE OF THE LEG (g) AND THE LOIN (g) AND THE MUSCULAR TISSUE OF THE CARCASS (g), AND REGRESSION EQUATIONS FOR PREDICTING THE WEIGHT OF CARCASS MUSCULAR TISSUE FROM THE MUSCULAR TISSUE OF THESE JOINTS

Dependent variate (weight of carcass muscular tissue in g) = Y

Carcasses	Independent variate = X	r	Regression equation	$s_{y.x}$ (g)
25 ewes	Muscular tissue of leg + loin	0.973	$Y = 3.695X + 1150$	463
	Muscular tissue of loin	0.927	$Y = 6.032X + 3280$	747
	Muscular tissue of leg	0.954	$Y = 8.013X + 287$	598
25 wethers	Muscular tissue of leg + loin	0.960	$Y = 3.569X + 1130$	530
	Muscular tissue of loin	0.855	$Y = 5.386X + 4431$	980
	Muscular tissue of leg	0.945	$Y = 7.300X + 620$	615
33 wether lambs	Muscular tissue of leg + loin	0.968	$Y = 3.975X - 228$	367
	Muscular tissue of loin	0.902	$Y = 7.099X + 1063$	629
	Muscular tissue of leg	0.944	$Y = 7.370X + 160$	480
11 wether lambs	Muscular tissue of leg + loin	0.975	$Y = 3.401X + 1060$	329
	Muscular tissue of loin	0.908	$Y = 6.625X + 1501$	619
	Muscular tissue of leg	0.966	$Y = 6.016X + 1813$	383
26 ewe lambs	Muscular tissue of leg + loin	0.988	$Y = 3.574X + 490$	217
	Muscular tissue of loin	0.942	$Y = 6.671X + 1207$	451
	Muscular tissue of leg	0.970	$Y = 6.742X + 772$	331

All correlations significant at the 1% level.

errors of estimate of the regression equations show that there is little difference between the combined leg plus loin bone weight and the leg alone in the accuracy of estimation of total bone in the carcass. When the standard error of bone weight for the leg plus loin was expressed as a percentage of the mean weight of bone in the carcass for each series of carcasses, the error in bone weight prediction ranged from 3.1% to 4.3%.

TABLE 5. Correlations between the Bone of the Leg (g) and the Loin (g) and the Bone of the Carcass (g), and Regression Equations for Predicting the Weight of Bone in the Carcass from the Bone of these Joints

Dependent variate (weight of bone in the carcass in g) = Y

Carcasses	Independent variate = X	r	Regression equation	$s_{y.x}$ (g)
25 ewes	Bone of leg + loin	0.955	$Y = 5.002X + 302$	100
	Bone of loin	0.838	$Y = 9.748X + 706$	185
	Bone of leg	0.948	$Y = 8.173X + 476$	108
25 wethers	Bone of leg + loin	0.923	$Y = 4.900X + 242$	131
	Bone of loin	0.778	$Y = 8.514X + 1059$	214
	Bone of leg	0.885	$Y = 7.616X + 463$	158
33 wether lambs	Bone of leg + loin	0.959	$Y = 5.063X + 5$	87
	Bone of loin	0.842	$Y = 11.48X + 177$	165
	Bone of leg	0.945	$Y = 7.460X + 268$	100
11 wether lambs	Bone of leg + loin	0.970	$Y = 4.953X + 73$	79
	Bone of loin	0.935	$Y = 9.437X + 462$	115
	Bone of leg	0.974	$Y = 9.700X - 187$	74
26 ewe lambs	Bone of leg + loin	0.972	$Y = 4.451X + 235$	57
	Bone of loin	0.897	$Y = 8.723X + 529$	107
	Bone of leg	0.974	$Y = 7.922X + 175$	56

All correlations significant at the 1% level.

Other Joints

Correlations have been presented in Table 6 between the total weight of the various components of 25 ewe carcasses and 33 wether-lamb carcasses and the same components in the neck, thorax, shoulder, and pelvis. In all cases the correlations are statistically highly significant. The tissues of the thorax joint alone appear to be as closely related to the same components of the whole carcass as was found to be the case for the leg and the loin (c.f. previous tables).

Discussion

Palsson (1939) suggested, for a number of reasons, that the leg and the loin were preferable to other anatomical joints as indices of the composition of the whole carcass. He considered that these two joints typified the whole carcass in rate of development since the leg is an early developing region while the loin is late developing. The two regions embrace the most valuable part of the meat animal and therefore he believed it was more important that they be studied in detail rather

TABLE 6. CORRELATIONS BETWEEN THE COMPONENTS OF THE NECK, THORAX, SHOULDER, AND THE PELVIS AND THE SAME CARCASS COMPONENTS OF 25 EWES AND 33 WETHER LAMBS

Variates	Ewes (25 pairs)	Wether lambs (33 pairs)
Carcass fatty tissue and fatty tissue of the neck	0.963	0.847
Carcass fatty tissue and fatty tissue of the thorax	0.988	0.983
Carcass fatty tissue and fatty tissue of the shoulder	0.965	0.972
Carcass fatty tissue and fatty tissue of the pelvis	0.942	0.955
Carcass muscular tissue and muscular tissue of the neck	0.919	0.935
Carcass muscular tissue and muscular tissue of the thorax	0.971	0.964
Carcass muscular tissue and muscular tissue of the shoulder	0.953	0.956
Carcass muscular tissue and muscular tissue of the pelvis	0.906	0.892
Carcass bone and bone of the neck	0.849	0.960
Carcass bone and bone of the thorax	0.959	0.952
Carcass bone and bone of the shoulder	0.837	0.968
Carcass bone and bone of the pelvis	0.949	0.918

All correlations significant at the 1% level.

than some low-priced part of the carcass. Furthermore, both these joints can be removed from the carcass with greater precision than most others and they are relatively easy to dissect.

Accuracy of jointing is a problem of greater or lesser magnitude involving all joints studied here. It is likely, however, that complete accuracy could never be achieved but, in any case, the general high order of the correlation coefficients between the various tissues of a sample joint and the same tissues in the whole carcass suggest that the accuracy of jointing was satisfactory.

Although it was shown that the thorax gives an equivalent or better index of carcass composition than the leg and the loin it is a difficult joint to remove from the carcass and is very time-consuming in dissection. On practical considerations therefore the thorax is an unsuitable joint. To a lesser degree the neck, shoulder, and pelvis are likewise unsuitable joints although of these three the pelvis is preferable in terms of ease of dissection and jointing. The thorax joint comprises approximately a quarter of the weight of the whole carcass (Table 2) and therefore it is not surprising that the weight of its components gives an accurate index of carcass composition and that it is a laborious joint to dissect.

From the point of view of the chemical composition of the carcass, Shorland *et al.* (1947) recommended that the loin be used as the representative sample joint, although they found that the chemical fat of the thorax was more closely correlated to the carcass chemical fat than any of the other joints analysed.

The present results derived from a larger number of ewe and wether carcasses and covering a wider range of age and weight than those of other workers confirm that the leg and the loin may be used as sample joints for accurately estimating carcass composition in terms of fat, muscle, and bone. It must be emphasised, however, that in spite of the high degree of relationship between the various dissectible components of the leg and of the loin and the same components in the carcass, caution must be taken in using sample joints in certain experiments. For instance, Preston and Gee (1957) and Kirton and Barton (in press) show that in sheep implanted with hexoestrol and thyroxine respectively there was a differential response evoked in these two joints by hormone treatment. If either joint had been used individually as a sample of the whole then it is highly probable that a different picture would have been presented for the effects of the treatments on the main tissues of the whole carcass.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial assistance of the Department of Scientific and Industrial Research covering various phases of the work. Emoluments provided by the New Zealand Wool Board and the Shell ~~Oil~~ Company to one of us (A.H.K.) are acknowledged.

The interest and guidance of Professor A. L. Rae is gratefully acknowedged.

REFERENCES

BARTON, R. A. 1947: Grading of New Zealand Fat Lamb Carcasses for Export. *Proc. 10th Ann. Meet. Sheepfarmers, Massey Agricultural College,* p.126.

CLARKE, E. A.; MCMEEKAN, C. P. 1952: New Zealand Lamb and Mutton. I. Anatomical Characteristics of Lamb and Mutton Carcasses. *N.Z. J. Sci. Tech. A33:* 1.

KIRTON, A. H.; BARTON, R. A. (in press): Liveweight Loss and its Components in Romney Ewes Subjected to *l*-Thyroxine Therapy and a Low Plane of Nutrition. I. Effects on Liveweight, Carcass Weight and Carcass Composition. *J. agric. Sci.*

PALSSON, H. 1939: Meat Qualities in the Sheep with Special Reference to the Scottish Breeds and Crosses *J. agric. Sci. 29:* 544.

PRESTON, T. R.; GEE, I. 1957: Effect of Hexoestrol on Carcass Composition and Efficiency of Food Utilization in Fattening Lambs. *Nature, Lond. 179:* 247.

SHORLAND, F. B.; DE LA MARE, P. B. D.; SORRELL, D. M. P.; BARNICOAT, C. R. 1947: Simplified Procedures for Determining the Nutritive Value of Carcasses with Special Reference to New Zealand Lamb and Mutton. *N.Z. J. Sci. Tech. A29:* 76.

DAMAGE IN RYEGRASSES BY *Hyperodes griseus* Hust.

By J. M. KELSEY, Entomology Division, Department of Scientific and Industrial Research, Ashburton

(*Received for publication, 12 May 1958*)

Summary

In New Zealand the Argentine wheat-stem weevil *Hyperodes griseus* is considered to be a more serious pest of ryegrass than it is of wheat. Its life history and habits in ryegrasses, and results of a three-year survey to assess damage are given in this paper.

INTRODUCTION

This weevil was first collected in New Zealand by D. Miller in 1927 (Marshall 1937), and recorded by Mr. L. Morrison in 1935 in wheat, barley, and ryegrass. Morrison (1938, 1939, 1949), and Hadfield (1952) record its presence in grasses in New Zealand. The present survey took place over the past three years, and was confined to three varieties of ryegrass. There were no stands where the weevil was absent; south Canterbury showed more damage than did mid and north Canterbury, and in all districts there were instances of complete destruction of first- and second-year stands. In general, the main losses were in thinning out of stands sometimes more or less uniformly but usually in patches of variable size.

TYPE OF DAMAGE

There are three types of damage caused by the weevil: (1) Feeding by adults gives a silvery appearance to ryegrass and other leaves. This type of damage occurred mainly to tips of leaves, but sometimes occurred as spots or strips on leaves, and in heavy weevil populations resulted in complete loss of leaves in local patches. Plant recovery from this type of damage is usually good if not complicated by larval damage. (2) The second and most severe type of injury is caused by larvae tunnelling in tillers. In young stands without seed heads the larvae moved downwards into crowns of plants, and it was found that if there was one larva per tiller, the result was death of the plant. Such injury resulted first of all in wilting of the plant, followed by browning, and at this stage superficial examination could mistake it for grass grub damage, as there were often extensive areas of dead pasture. In the case of the weevil damage however, unlike that of the grass grub, the root system was perfectly sound. A second period during which larvae sometimes caused death of plants was after harvesting had been completed, when larvae moved down into crowns and the present survey indicated that a great deal of the "non-recovery after harvesting" of ryegrass stands was due to injury by *H. griseus* larvae. (3) The third type of injury is to seed heads. When seed heads were being produced, larvae nearly always moved upwards in stems and the result was a "white head" that did not set seed. This type of injury was not important in so far as seed yields were concerned, as the most severely affected paddock had only 6.9% of total straws affected in this way, but populations of weevil were often so high that it may be possible for severe injury to occur if heavy larval populations coincided with the pre-flowering stage of seedhead production.

LIFE HISTORY

H. griseus overwintered as adults, but the latter were found during any month of the year. There was no hibernation in the true sense as adults were observed to feed on a variety of plants throughout winter months when warm, sunny weather was experienced. In spite of all-the-year-round presence of adults, there were two clearly marked generations in each of the three years of the survey. The earliest date for presence of eggs in the field was the last day of August, when three eggs were seen in one of 450 plants examined.

Throughout September, October, and as late as 8 November eggs were present. Larvae were present from the last week in September until mid December. Pupae were present from the last week in November till mid January, and adults from these appeared from the beginning of December till end of February. Eggs of the second generation were present from 17 December till end of March; larvae from mid January to beginning of May: pupae from the beginning of March to mid May. Eggs took 12 24 days to hatch in the laboratory, but apparently took slightly longer in the field where no larvae were recorded earlier than 23 days from oviposition. Pupal period varied from 7–15 days.

Habits

Adult beetles were active both by day and night, and fed during the day when the sun was obscured by clouds, but they were mainly nocturnal so far as feeding was concerned. Mating flights occurred during bright sunlight and were noted from near sea level to over 4,500 ft in tussock areas. Beetles were difficult to detect in or on the soil because, quite apart from their light- and dark-brown natural colouration, in moving through soil they often became coated with soil which adhered to the short stout bristles and scale on the elytra and prothorax. In addition, beetles when disturbed "played possum" for seldom less than 20 minutes, and up to 45 minutes, with the result that they resembled small particles of soil. In heavy infestations, populations of over 28 per square foot were found by slowly flooding soil samples from below in trays in the laboratory, but in the field it was not often that more than two adults could be observed in the same sample. If an average of 1–2 per square foot can be seen during the day, the actual infestation is a severe one and probably over 20 per square foot. Adults fed usually only on one surface of grass leaves, but left the upper or lower cuticle with the result that attacked leaves possessed the typical silvery appearance. (Rather similar to that due to slug damage.)

During oviposition (in moist, fleshy tissue that was usually green, but occasionally brownish) adults made a pocket in leaf sheath tissue always from the back of leaf sheaths where they closely adhered to

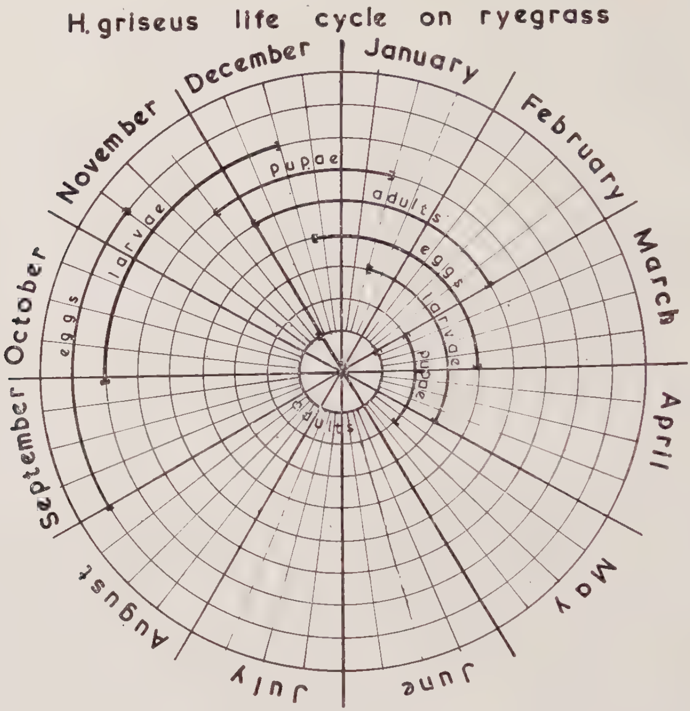


Fig. 1.—Diagrammatic illustration of the life-cycle of *H. griseus* on ryegrass.

tillers. These pockets never extended through to the next leaf or stem and no eggs were laid between sheath and stem. In each pocket only one aperture was made, and two greenish-black, bean-shaped eggs were laid usually end to end, but occasionally single, or three eggs per pocket were found. Most eggs were inserted from $\frac{1}{8}$–$\frac{1}{4}$ in. below leaf "collars" but occasionally up to 1 in. below, and though many eggs were found in leaves closest to the ground some were as high as the 6th leaves from the crown of the plant, though this was still not more than 2 in. from ground level.

When eggs hatched, the larvae always tunnelled in leaf sheaths for a while, and always made their way downwards. After this, however, there was no consistency about larval behaviour; some remained in leaf sheaths until they reached points of attachment to tillers, and there completed development; others, after a short distance in leaf sheaths, tunnelled directly into centres of tillers between nodes; still others entered tillers at or just above the nearest nodes, and some ringed outside of nodes. Larvae that entered at one node almost invariably remained in that internode for plants producing seed heads (two exceptions only in 3,800 straws), but in young plants, larval movement was nearly always downwards into crowns of plants irrespective of presence or absence of nodes (seven exceptions in nearly 6,900 tillers). In presence of seed heads, larvae entered near any of the nodes from

2nd–7th from the crowns, and though initially they moved upwards they usually moved down again as they approached the fully fed state. Any site of tunnels caused "white heads" since fully grown larvae were almost the same width as internal stem diameters, and the same thing arose for larvae in young plants, the effect of larval activity being "ring barking" from inside. When fully fed, larvae cut holes in sides of tillers or straws and dropped to the ground where they sought shelter under plant cover. Pupae were seldom found in the soil at depths greater than ¼ in.

Host Plants

Eggs were laid in, and larvae fed on, short-rotation (H1), Italian and perennial ryegrasses, cocksfoot, sweet vernal, Chewings fescue, wheat, oats, and barley, and also in petioles of young rape plants, though larvae were not seen to tunnel extensively in rape. Adult beetles fed on all grasses except Yorkshire fog, on clovers, lucerne, cereals, and cruciferous crops.

ECONOMICS

Samples from five different paddocks throughout Canterbury were examined in each of the years 1955 and 1956, and six in 1957; the age of ryegrass stands being from 2 months to 3 years. In each paddock 50 samples (each a complete plant) were taken at intervals of 20 paces in two parallel rows dividing the area into approximate thirds. Samples were examined and dissected under the 7× microscope. The average number of tillers per plant was 6.9 (range 3–11) for young stands, and

TABLE 1. *H. griseus* INFESTATION IN RYEGRASSES
(Based on 50 plants per paddock)

Area (acres)	Approx. Age (months)	Variety of Ryegrass	State at Which Infested	Total No. Tillers	% Damaged
10	2	Italian	Vegetative	365	89.8
23	3	,,	,,	342	74.5
17	3	,,	,,	422	97.8
26	12	,,	,,	457	45.9
32	12	,,	Seeds heads	731	1.5
19	24	,,	,, ,,	681	1.7
35	9	Perennial	Vegetative	652	35.4
18	24	,,	Seed heads	498	1.8
14	2	H1	Vegetative	427	84.5
11	2	,,	,,	459	97.3
15	3	,,	,,	335	57.6
25	4	,,	,,	420	94.7
16	12	,,	,,	675	87.4
18	12	,,	Seed heads	653	6.2
31	36	,,	,, ,,	563	3.0
41	36	,,	,, ,,	718	6.9

8–15 for older stands. Damage was assessed on the basis of how many tillers were damaged beyond recovery. The following table gives results of the survey over three years:—

Table 2 gives results of visual assessment of *H. griseus* damage to ryegrass areas not showing seed heads. Figures are based on larval damage in an area of 1 square yard at each of 100 inspection points 20 paces apart in four parallel rows each of 25 inspection points: the paddocks were divided into approximate fifths.

TABLE 2. VISUAL ASSESSMENT OF *H. griseus* DAMAGE BASED ON NUMBERS OF PLANTS KILLED IN 1 SQ. YD AT 100 SAMPLING POINTS AT 20-PACE INTERVALS

Area (acres)	Approx. Age (months)	Variety of Ryegrass	% Damaged	
14	3	Italian	60	
32	4	,,	100	
22	12	,,	25	
16	12	,,	40	
35	12	,,	100	Average 65%
15	4	Perennial	45	
10	12	,,	30	
20	12	,,	40	
27	12	,,	35	Average 37.5%
11	3	H1	55	
27	3	,,	40	
17	4	,,	95	
37	4	,,	50	
11	4	,,	100	
18	12	,,	30	
34	12	,,	60	
24	24	,,	50	Average 60%

DISCUSSION

From data in these tables it is apparent that *H. griseus* damage can be very severe, but the areas on which damage assessment was carried out do not represent a true average cross section of all ryegrass stands throughout Canterbury. Nearly all cases were those on which farmers sought information, and the remainder were in close proximity to these, and it would be only very observant farmers, or those with very badly damaged pastures who would notify the Division when pasture troubles arose. It is considered, therefore, that the paddocks assessed were typical of what occurs with the worst *H. griseus* infestations. In the paddocks examined over the three-year survey period there was more damage to short-rotation and Italian strains than to perennial ryegrass, and in randomised 1/40 acre plots at Grasslands Division, Lincoln, examined in March 1957 there was an almost complete elimination of short-rotation in all four replications, but not in perennial plots. There was not sufficient data to indicate any preferential selection between short-rotation and Italian ryegrasses. Damage to seed heads always occurred most severely in the thinnest parts of ryegrass stands, and it was found that this was due to a carry-over of weevil from initial infestation during the early stages of grass establishment. The most severe seed damage (6.9% of total straws) occurred to short-rotation ryegrass, and though this is not a very severe loss, the writer considers that the incidence of *Hyperodes* damage is increasing year by year, and this phase of damage should be closely watched. Mr. D. Wraight of the Department of Agriculture, Timaru, supplied seed cleanings from 22 acres of short-rotation ryegrass at Pareora harvested in 1955-56 season; in the seed cleanings from 363½ bushels (= 330.4 lb per acre) of dressed seed from this area were 140 lb of material of which 78% (109 lb) was *Hyperodes* adults. There was an average of 192,320 weevils per pound so there were 20,962,880 weevils in the 109 lb of weevils from this particular paddock; an average of 952,858 per acre or 21.1 adult weevils per square foot. As stated earlier in this paper, a population of over 20 adults per square foot can result in total loss of a ryegrass stand due to larval damage by the next generation of *H. griseus.* The average production of machine-dressed short-rotation ryegrass seed in 1956 was 596.3 lb per acre (Anon. 1957) so there was a reduction in the Pareora yield of 265.9 lb per acre.

CONTROL

No intensive campaign has been conducted to find an economic method of preventing stem-weevil damage, but laboratory tests showed that both DDT and lindane would control adults.

The habits of the weevil indicate that insecticides would, in all probability, have to be applied against each of the two generations, because the weevil normally does not at any stage enter soil to a depth greater than ¼ in., and most insecticides would be washed fairly rapidly by rain beyond this point. Also, reinfestation of a given area by beetles flying or moving in from adjacent paddocks appears to be fairly rapid.

Rotation of crops as a remedial measure does not give much promise of success as adults are general feeders on all cruciferous crops, most grasses, clovers, and lucerne, and also on all cereals. The only possibility appears to be to grow a potato crop after cereals and crucifers before sowing ryegrasses.

REFERENCES

ANON, 1957: *New Zealand Official Year Book 1957,* p. 527.

HADFIELD, J. W. 1952: Arable Farm Crops of New Zealand. *N.Z. Dep. sci. industr. Res. Inf. Ser. No. 5:* 49-50.

MARSHALL, GUY A. K. 1937: New Curculionidae (Col.) in New Zealand. *Trans. roy. Soc. N.Z. 67:* 316-40.

MORRISON, L. 1935: Pests of the Wheat Crops. *Canterbury Agric. Coll. Lincoln Bull. 71:* 1-2.

——————1938: Surveys of the Insect Pests of Wheat Crops in Canterbury and Nr. Otago during the Summers of 1936-37 and 1937-38. *N.Z. J. Sci. Tech. A20:* 142-55.

——————1939: Insect Pests of the Wheat Crops. *N.Z. Wheatgrower 9 (6):* 9-13.

MORRISON, L.; BLAIR, I. D. 1949: Wheat Diseases and Insect Pests. *N.Z. Dep. sci. industr. Res. Inf. Ser. No. 3:* 37-40.

THE EFFECT OF MAGNESIUM TOPDRESSINGS ON THE GROWTH OF YOUNG APPLE TREES

By H. O. ASKEW and E. CHITTENDEN, Cawthron Institute,
Nelson

(*Received for publication, 22 May 1958*)

Summary

Young Sturmer apple trees planted on soil low in magnesium showed severe premature defoliation and were seriously retarded in growth.

Soil applications of magnesium corrected this deficiency and produced normal healthy growth.

INTRODUCTION

Magnesium deficiency in apple trees, causing premature defoliation, has been widespread in the main apple-growing districts of Nelson. The soils associated with this mineral deficiency are Mapua clay loam, a hill soil formed on Pleistocene gravels, and Motupiko loam, an alluvial soil of the valleys derived from the same parent material.

In the original work on the control of this deficiency (Kidson, Askew, Chittenden 1943) it was found that effective control was obtained from the use of 10 cwt of dolomite per acre every third or fourth year. In this earlier work attention was paid to mature trees but it was found in later years that magnesium deficiency could severely affect young trees not only causing defoliation but also substantially affecting their rate of growth.

In this paper a comparison is given of the growth of two young Sturmer trees, one treated with magnesium and the other without magnesium, for the first four years of their growth. Observations then ceased owing to a mishap to the trees.

EXPERIMENTAL

The orchard on which this trial was made had been used since 1939 for field work in connection with magnesium deficiency. By 1947 the control trees had deteriorated to such an extent that it was decided to treat some of them with dolomite and magnesium sulphate. Some of the trees recovered and grew vigorously but others had become too weak and died. Consequently in 1951 two young Sturmer trees on Northern Spy stock were planted to replace dead trees, one on a magnesium treated area and the other on an untreated area.

The treated area had received two applications of magnesium prior to the trees being planted, and another application was given in 1952, as follows:

1947—12 lb dolomite + 7 lb magnesium sulphate per tree.

1949— 6 lb dolomite per tree.

N.Z. J. agric. Res. 1: 796–799

1952— 6 lb dolomite per tree.

A standard NPK fertiliser mixture was given annually to both areas.

An analysis of the soil from the two sites is shown below:

TABLE 1. SOIL ANALYSIS OF MOTUPIKO LOAM

	Depth of Sampling (Inches)	pH	B.E.C. (m.e.%)	T.E.B. (m.e.%)	Base Satn.%	Exchangeable Bases (m.e.%)		
						CaO	MgO	K_2O
Untreated	0– 6	5.0	9.6	0.70	7	0.75	0.11	0.58
	6–12	4.9	7.6	0.13	2	0.28	0.14	0.36
Magnesium treated	0– 6	5.4	9.6	5.62	59	4.09	1.47	0.71
	6–12	5.1	7.3	0.65	9	0.88	0.50	0.20

Dolomite applications have been effective in raising the levels of magnesium and calcium, particularly in the topsoil, with a corresponding rise in the pH value.

It might be thought that the low calcium figures on the untreated soil may have had some effect on retarding growth but applications of limestone without magnesium in earlier trials only worsened the condition of the trees (Kidson, Askew, Chittenden, 1943).

In the year after planting (1952) the control tree was showing severe defoliation while the foliage on the treated tree was quite healthy. There was little difference in their size at this time. In the following three years however the difference in their growth progressively widened.

Measurements of tree height taken in 1954 and 1955 showed as follows:

1954—Mg-treated 5 ft 10 in; untreated 4 ft.

1955—Mg-treated 6 ft 10 in; untreated 4 ft 6in.

A comparison of their progress is best shown by the accompanying photographs which were taken each year from 1952-55 inclusive and record their relative size in each of the four years.

In Fig. 1 (1952), although the trees are of the same height at this time, the untreated tree has lost most of its foliage while that on the Mg-treated tree shows normal healthy growth.

Fig. 2 (1953) shows the treated tree gaining in size, with good foliage and strong lateral growth, while the untreated tree is again defoliated and has weak spindly limbs.

Figs. 3 and 4 (1954 and 1955) show the differences in the size of the trees increasing. The treated tree has grown as would be expected under normal conditions while the tree with no magnesium is small and weak limbed and has lost most of its foliage.

ACKNOWLEDGEMENTS

The authors wish to thank Messrs. E. J. and N. J. Williams, Lower Moutere, for the use of their orchard in the conduct of these experiments, and Mr. L. Hodgson for chemical analysis of the soils.

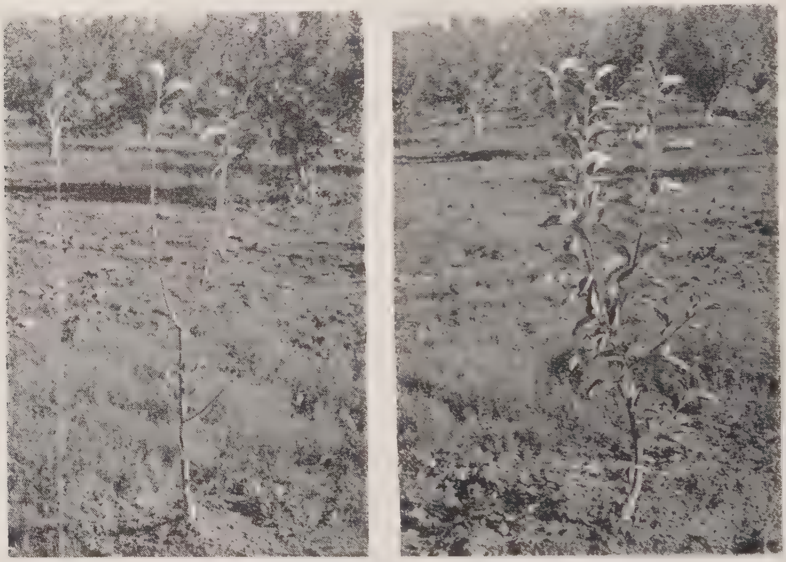


Fig. 1.—1952: *Left* Untreated. *Right* Treated.

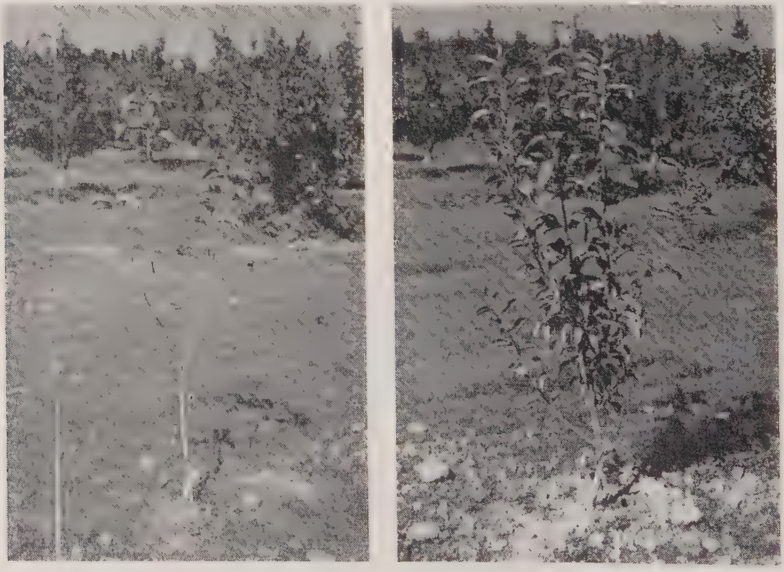


Fig. 2.—1953: *Left* Untreated. *Right* Treated.

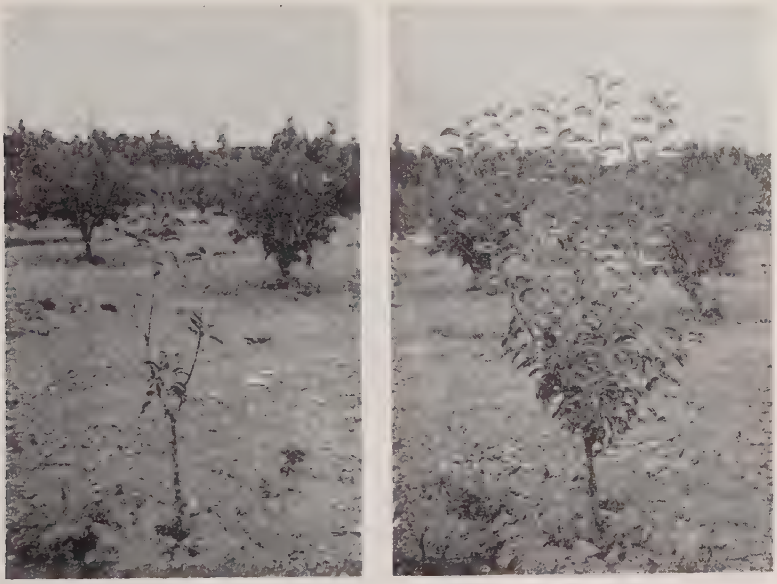


Fig. 3.—1954: *Left* Untreated. *Right* Treated.

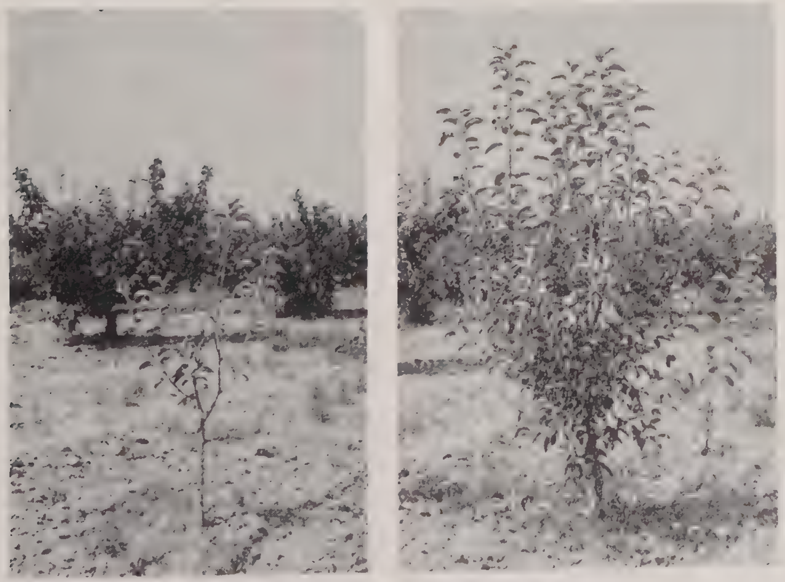


Fig. 4.—1955: *Left* Untreated. *Right* Treated.

REFERENCE

KIDSON, E. B.; ASKEW, H. O.; CHITTENDEN, E. 1943: *N.Z. J. Sci. Tech. A25*: 31-42.

FUNGAL LEAF-SPOT DISEASES OF COCKSFOOT (*Dactylis glomerata* L.) IN THE MANAWATU

591

II. PURPLE LEAF-SPOT CAUSED BY *Stagonospora maculata* (Grove) Sprague, AND LEAF FLECK CAUSED BY *Mastigosporium rubricosum* (Dearn et Barth.) Sprague.

By H. T. WENHAM and G. C. M. LATCH*, Massey Agricultural College, Palmerston North.

(*Received for publication 16 April 1958*)

Summary

Stagonospora maculata and *Mastigosporium rubricosum* are recorded as causing leaf-spot diseases on cocksfoot in the Manawatu.

Disease symptoms, morphology, colony characteristics on media, and manner of spore germination are described.

Neither disease is of economic importance in New Zealand.

MATERIAL AND METHODS

The pathogens were isolated from small pieces of diseased tissue approximately 10 mm square cut from young lesions. The tissue pieces were first rinsed in 70% ethyl alcohol for 30 seconds, transferred to a 0.001% solution of mercuric chloride for 90 seconds, and then thoroughly washed in three changes of sterile water. After drying, the pieces were set out on petri dishes of potato dextrose agar (P.D.A.) and incubated at 23°c. When colonies had grown sufficiently for positive identification transfers were made to agar slopes in test tubes.

The inoculum used for pathogenicity tests was prepared by adding 15-day-old colonies to flasks of sterile water and thoroughly agitating to give a suspension of conidia and mycelial fragments. Healthy plants in pots of sterilised soil were sprayed with this suspension, held in a humidity cabinet for 48 hours and then removed to a glasshouse the temperature of which ranged between 15° and 22°c. Control plants were given identical treatment except that they were sprayed with sterile water.

The methods adopted for study of morphology, colony characteristics on media, the effect of temperature and media pH on growth rate, and manner and rate of spore germination, were the same as previously described for *Scolecotrichum graminis* Fckl. (Latch and Wenham 1958).

Purple Leaf-spot Caused by Stagonospora maculata *(Grove) Sprague.*

The Disease

Lesions are first apparent on leaves in late November as minute watery spots. They darken rapidly to a purplish-black colour, may

---

* Present address: Plant Pathology Dept., University of Wisconsin, Madison 6, Wisconsin, U.S.A.

N.Z. J. agric. Res. 1: 800–808.

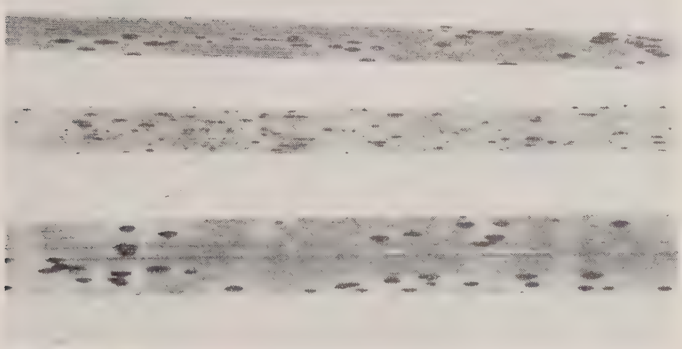


Fig. 1.—Lesions caused by *S. maculata* on leaves of cocksfoot.

elongate up to 5 mm in length but rarely coalesce (Fig. 1). The causal fungus has also been isolated from lesions on sheaths and culms. Early stage symptoms may at first be mistaken for those of leaf-streak caused by *Scolecotrichum graminis* Fckl. When mature they are still relatively small, however, whereas those of leaf-streak elongate greatly. Further, mature lesions of leaf-streak disease have a lighter central area whereas those caused by *S. maculata* remain purplish-black, from which feature is derived the common name of purple leaf-spot disease.

Both Sprague (1950) and Graham (1952) refer to golden-brown or black pycnidia of the causal fungus on naturally diseased leaves. Although in the present study the disease was under observation for two years during which time scores of lesions of all ages were microscopically examined, in no instance were pycnidia observed. Likewise pycnidia were never found associated with lesions on artificially inoculated plants.

S. maculata has been reported as pathogenic to species of the genus *Dactylis* only. In using 12 species of grasses in 10 genera for glasshouse inoculation tests, Graham (1952) found *Dactylis glomerata* L, and *D. aschersoniana* Graebn. to be susceptible. In the United States the disease is considered important in pastures in the Middle Atlantic States (Sprague 1948) and as the major disease of cocksfoot in the North-East (Graham 1952). In the Manawatu, purple leaf-spot first appeared in late November, was widespread throughout the summer and autumn but could not be located after mid May. At no stage was it of economic importance.

The Causal Fungus

Taxonomy. Stagonospora subseriata (Desm.) Sacc. is a foliage pathogen of a number of grass species (Sprague 1950). A distinct form was found on *Dactylis glomerata* L. by Grove (1935) where the pycnidia were arranged in lines on sub-elongated blackish brown blotches, but having spores similar to *S. subseriata*. This variant form he named *S. subseriata* var. *maculata* (Grove). Sprague (1941) examined a number of American collections from cocksfoot and concluded they were identical with Grove's form. He described the spores as fusiform, 3- to 4-septate, yellow with coarse contents, 27 to 40 by 4.8 to 6.5μ (av. 33 by 5.5μ), and borne in globose golden-brown pycnidia with walls about 5μ thick. Later Sprague (1948) ruled that differences in morphology and pathogenicity between *S. subseriata* and *S. subseriata* var. *maculata* were of sufficient magnitude to warrant elevating the latter fungus to specific rank, and proposed the name *S. maculata* (Grove) Sprague.

Cultural Features and Sporulation. Of the 9 media tested, growth was most rapid on cornmeal agar, P.D.A. and cocksfoot agar, least rapid on soya-bean agar, water agar, carrot agar and malt agar. At 11 days colonies on P.D.A. were 57 mm in diameter and consisted of a dense slightly raised mycelial mat with short white aerial hyphae (Fig. 2). Most colonies developed a deep-pink or red colouration at the centre. Pycnidia were frequently associated with such areas in colonies 3 to 4 weeks old. It was found that the time taken for production of pycnidia could be shortened by exposing 5-day-old cultures to ultra-violet light at a distance of 15 cm for 5 minutes. Following this method, pycnidia were produced within 8 to 12 days on P.D.A., cornmeal agar, oatmeal agar, tomato agar, malt agar and carrot agar, and on sterile cocksfoot and pea stems. The extent to which pycnidia were produced depended greatly upon the isolate, many cultures being sterile. Pycnidia produced on P.D.A. were black, readily discernable to the naked eye, and ranged in size from 215 to 375μ (av. 285μ). Spores were straight or slightly curved, 2- to 7-septate (av. 3), with very granular contents. The spores were usually pointed at the tip and blunt at the base. The dimensions of 300 spores from 14-day-old P.D.A. cultures held at 24°c were 26.5 to 44.5μ by 4.5μ (av. 36.6 by 4.5μ).

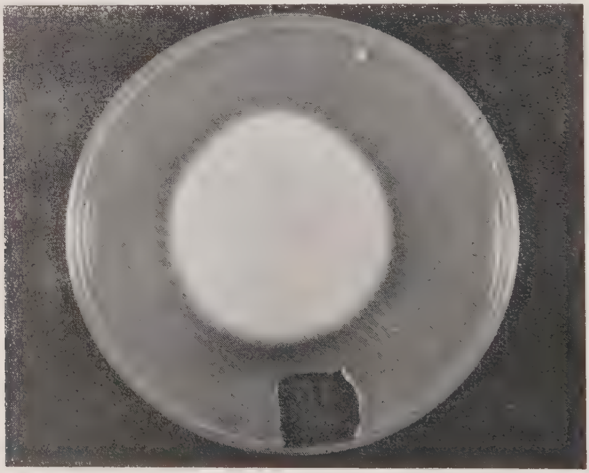


Fig. 2.—An eleven-day-old culture of *S. maculata* grown at 24°c on P.D.A. Colony diameter 57 mm.

The fungus grew on P.D.A. held at temperatures between 5° and 32°c. Growth was most rapid between 20° and 28°c, with an optimum at 24°c. Growth occurred on P.D.A. at all pH values tested between 3.0 and 9.0. Optimum growth occurred at pH 5.9, colonies averaging 45 mm after 9 days at 24°c. At lower pH values the development of red pigmentation was most pronounced.

Spore Germination. Tests were made on P.D.A. and in distilled water at temperatures ranging from 7° to 32°c. The optimum temperature for germination was between 24° and 28°c, with strong growth at 32°c. At 6°, 15° and 18°c, a gradual increase in growth rate was noted, although at each temperature there were individual spores with germ tubes of much greater length than the average for that temperature. A characteristic feature was that a few spores germinated well ahead of the majority at each temperature.

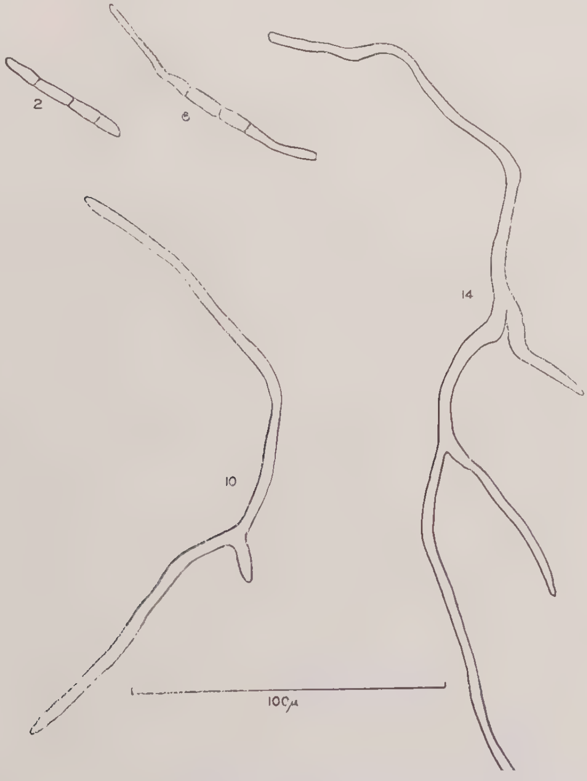


Fig. 3.—Stages in germination of *S. maculata* coridium on P.D.A. at 24°c over a 24-hour period.

Both on P.D.A. and in distilled water, germination began with the development of one or two germ tubes emerging terminally from the spore. These continued to elongate for 10 to 12 hours before branching, by which time the limits of the original spore were lost (Fig. 3).

Leaf Fleck Caused by Mastigosporium rubricosum *(Dearn et Barth.) Sprague*

The Disease

Infected leaves develop numerous dark-brown to purple flecks each surrounded by a water-soaked area. With age, the lesions enlarge up to 12 by 5 mm (Fig. 4). Mature lesions are almost black with a thin yellow-brown halo, while most have a central cream-coloured area from which a single crop of spores is produced from short conidiophores. Microscopically the conidia appear as a silvery mass which could be mistaken for dead cuticle splayed back by the pathogen. Conidial masses are best seen on fresh lesions in the early morning when dew is present. On severely diseased leaves dead areas may coalesce. Lesions caused by *M. rubricosum* are similar to those of *Stagonospora maculata*, especially in the younger stages. As the lesions increase in size, the symptoms may be correctly identified since those of the former are light in colour at the centre whereas lesions caused by *S. maculata* remain dark.

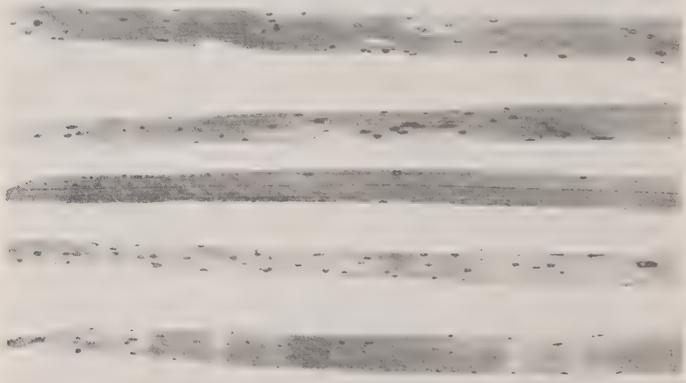


Fig. 4. Cocksfoot leaves showing field symptoms of leaf-fleck disease.

M. rubricosum is widespread in Europe and North America where it is reported to cause severe damage to a number of grasses (Stapledon and Jenkins 1922; Sampson and Western 1954; Bollard 1950; Sprague 1950; Creelman 1956). On *Dactylis glomerata* L. the fungus caused a serious leaf-spot disease in a number of countries (Sprague 1938, 1946; Sampson and Western 1954, Bollard 1950). In the Manawatu, leaf fleck was found to have a very limited season, diseased leaves being found only from mid August to early November.

Sprague (1950) lists twelve hosts of *M. rubricosum* of which seven are *Agrostis* species. Four new hosts have since been listed by Sprague (1955) all of which were found in Alaska.

The Causal Fungus

Taxonomy. Species of the genus *Mastigosporium* are known only as parasites causing leaf-spot diseases of grasses. *M. album* Riess. is pathogenic to a number of grasses and is characterised by 4– to 5–septate conidia with one or more filiform appendages at their distal end. Davis (1903) described a non-appendaged form which he named *M. album* var. *calvum* Davis. Sprague (1938) studied this form in the United States and elevated it to species rank, naming it *M. calvum* (Davis) Sprague. Dearness (1917) recorded *Fusonia rubricosa* Dearn. et Barth., as a new foliage pathogen of cocksfoot in North America, and Wakefield (1918) reported *M. album* var. *muticum* Sacc. as a new British fungus also on cocksfroot. However, from the description of spore morphology Sprague (1938) considered these two fungi to be identical with his *M. calvum.* Later Sprague (1940) renamed the fungus *Mastigosporium rubricosum* (Dearn. et Barth.) Sprague to conform with the International Rules of Botanical Nomenclature which state that varietal names raised to specific rank are not valid when a specific epithet is available.

Bollard (1950) conducted a study of the four known species of *Mastigosporium* attacking grasses and, on the basis of distinct differences in morphology, cultural features, and pathogenicity, designated the fungus attacking *Agrostis stolonifera* L. as a new variety, *Mastigosporium rubricosum* var. *stolonifera* Bollard. This form he found to be pathogenic to species of *Calamagrostis* but not to *Dactylis glomerata* or other species of *Agrostis.* He further proposed that the name *M. rubricosum* be retained for the form attacking *Dactylis glomerata,* which he states is also pathogenic to *D. aschersoniana,* but not so to species of *Agrostis.*

Morphology. A single crop of conidia are produced on lesions by expansion and abscission of the distal portion of short, continuous, erect conidiophores. For spore-measurement studies it was found that the best time to collect conidia was early morning with dew still on the lesions. Alternatively, leaves with very young lesions were subjected to periods of high humidity in petri dishes. The conidia were hyaline, elliptical, generally 3-septate with constrictions at the septa (Fig. 5). A few spores were 2- or 4-septate. The dimensions of spores from

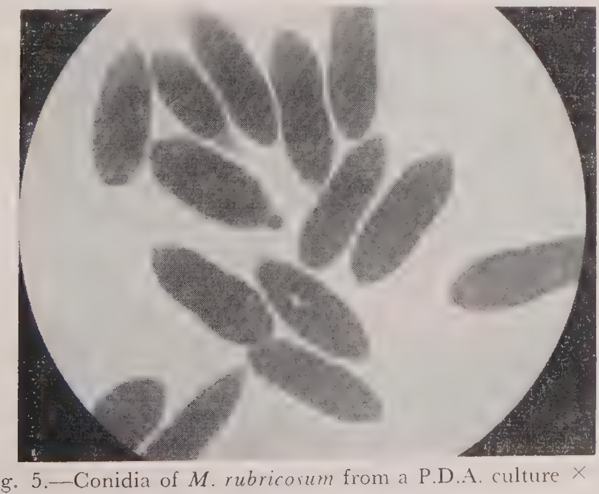


Fig. 5.—Conidia of *M. rubricosum* from a P.D.A. culture × 450.

naturally diseased leaves ranged from 38 to 52.8μ (av. 45.3μ) by 11.6 to 16.5μ (av. 15.6μ). The length of spores from *D. glomerata* are recorded by Bollard (1950) as 31 to 59μ (av. 42.74μ), and by Sprague (1950) as 29 to 60μ.

Cultural Features and Sporulation. M. rubricosum is slow-growing on agar media. Colonies on P.D.A. held at 13°c consist of a creamy-yellow mat of mycelium of a tough leathery texture, the central portion of which rises to a brown shiny mass of conidia. After nine days growth, slight convolutions of the central raised area develop, which daily become more pronounced (Fig. 6). On the majority of media tested the above description holds true, apart from minor differences in colour and texture of the mycelium. Growth was most rapid on oatmeal agar, cornmeal agar, tomato agar, and P.D.A, least rapid on carrot agar, soya-bean agar, and water agar.

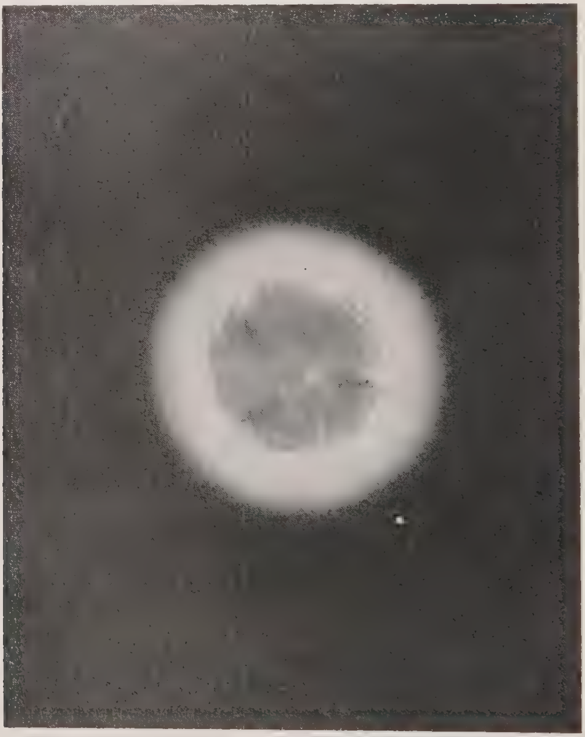


Fig. 6.—Culture of *M. rubricosum* on P.D.A. held at 13°c for 21 days. Colony diameter 17.5 mm.

Bollard (1950) found the maximum and optimum temperatures for growth on cocksfoot agar to be 25° and 15°c respectively. In the present study, slight growth occurred on P.D.A. at all temperatures tested between 5° and 28°c, with optimum growth at approximately 12°c. At 24°c, colonies were yellow to off-white in colour, greatly raised from the agar surface, with few spores. Growth rate appeared to be independent of hydrogen ion concentration. Colonies at the higher pH values developed much greater numbers of conidia. Apart from differences in conidial concentration, colony features remained very similar at the different pH levels.

The optimum temperature for spore germination was in the vicinity of 19°C. At this temperature the appearance of germ tubes was preceded by a swelling of the spore, usually within 3 to 4 hours of preparing the spore suspension. Germ tubes developed from any cell of a spore. They usually branched profusely within 12 hours, were irregular in shape and were characterised by the presence of large vacuoles (Fig. 7).

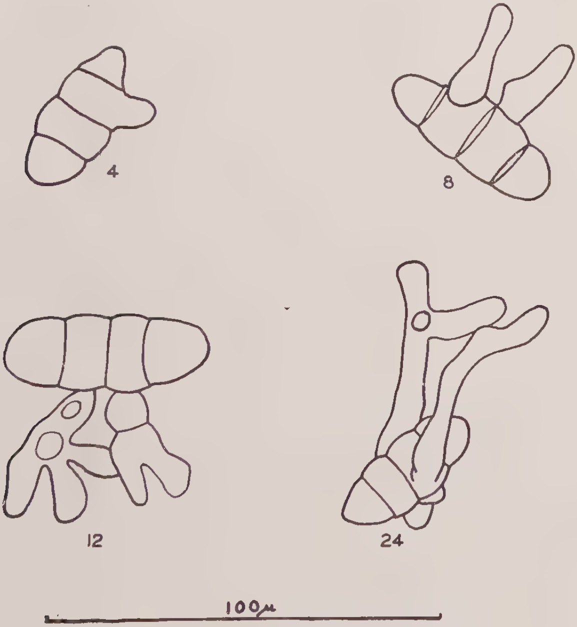


Fig. 7.—Stages in germination of *M. rubricosum* conidium in distilled water at 19°C over a 24-hour period.

REFERENCES

BOLLARD, E. G. 1950: *Trans. Brit. mycol. Soc. 33:* 250-64.

CREELMAN, D. W. 1956: *Plant Dis. Reptr. 40:* 510-2.

DAVIS, J. J. 1903: *Trans. Wis. Acad. Sci., Arts, Lett. 14:* 83-106.

DEARNESS, J. 1917: *Mycologia 9:* 345-364.

GRAHAM, J. H. 1952: *Phytopathology 42:* 653-6.

GROVE, W. B. 1935: "British Stem and Leaf Fungi." C.U.P.

LATCH, G. C. M.; WENHAM, H. T. 1958: *N.Z. J. agric. Res. 1:* 182-8.

Sampson, K.; Western, J. H. 1954: "Diseases of British Grasses and Herbage Legumes." C.U.P.

Sprague, R. 1938: *J. agric. Res. 57:* 287-99.

——————1940: *Mycologia 32:* 43-5.

——————1941: *Ibid. 33:* 371-9.

——————1946: *Plant Dis. Reptr. Suppl.* p. 163.

——————1948: *Mycologia 40:* 177-193.

——————1950: "Diseases of Cereals and Grasses in North America." Ronald Press, New York.

——————1955: *Plant Dis. Reptr. Suppl.* p. 232.

Stapledon, R. G.; Jenkins, T. J. 1922: *Bull. Welsh Pl. Breed. Sta. Ser. H. 1:* 47.

Wakefield, E. M. 1918: *Kew Bull. 6:* 229-33.

NEW ZEALAND JOURNAL OF SCIENCE

Vol. 1 No. 3, September 1958

CONTENTS

The Contamination of Bacon by Arsenic from Smoke Derived from Preservatised Wood. *C. C. Watson*

The Contamination of Fish by Arsenic from Smoke Derived from Preservatised Wood. *C. C. Watson*

Mercury Content of Tomatoes. *H. M. Stone* and *P. J. Clark*

Determination of Diphenylamine Residues on Apples *Helen E. Harvey*

Notes on Settlement in *Lepas* Barnacles. *T. M. Skerman*

The Suspended Plummet Method for Density Measurements. *A. E. Gilmour.*

Rates of Growth in Two Species of *Lepas* (Cirripedia). *T. M. Skerman*

The Detection of Food Preservatives by Paper Chromatography. *Alison C. Cooke*

The Collection of *Hylemyia seneciella* (Meade) (Diptera, Muscidae) for Shipment to Australia. *J. M. Hoy*

A Note on the Determination of Low Concentrations of Sulphate in Rain and Other Waters. *J. K. Johannesson*

The Application of Table Flotation to the Removal of Sulphides from a Scheelite Concentrate. *M. H. Buckenham* and *F. L. Sanderson*

Apparatus for Investigating Spatial Distribution of Plankton. *R. M. Cassie.*

Contributions to the Quaternary History of the New Zealand Flora. Part 1. Auckland Island Peat Studies. *N. T. Moar*

Notes on the Botany of the Auckland Islands. *N. T. Moar*

Contributions to the Quaternary History of the New Zealand Flora. Part 2. Plant Remains from a Buried Peat Layer at Bowenwater, Christchurch. *N. T. Moar*

CONTENTS

PAGE

Effectiveness of Honey Bees and Bumble Bees in the Pollination of Montgomery Red Clover. *I. W. Forster* and *W. V. Hadfield* 607

Effect on Honey Bees of Some Defoliants. *T. Palmer-Jones* and *I. W. Forster* 620

Effect on Honey Bees of DDT Applied from the Air as a Spray to Lucerne: Notes on Lucerne Polination. *T. Palmer-Jones* and *I. W. Forster* 627

Specific Gravity as an Index of the Fat Content of Mutton Carcasses and Various Joints. *A. H. Kirton* and *R. A. Barton* 633

Studies on the Biology of Peach Rust (*Tranzschelia pruni-spinosae* Pers.) in New Zealand. *Hasan Jafar* 642

Some Effects of Soil Physical Condition on Ryegrass Growth. *D. B. Edmond* 652

Investigation on the Control of Peach Rust (*Tranzschelia pruni-spinosae* Pers.). *Hasan Jafar* 660

Some Long-term Effects of Fertilisers on a Mown Turf of Browntop and Chewing's Fescue. *D. B. Edmond* and *S. T. J. Coles* 665

The Effect of Different Phosphate Fertilisers on Soil pH and the Consequent Effect on Phosphate Retention. *W. M. H. Saunders* 675

Studies of Grazing of Crop Forages by Sheep. II. Wastage of Rape by Grazing Wethers. *A. F. Greenall* 683

Effect on Honey Bees of Toxaphene and Strobane Applied to White Clover Pasture. *T. Palmer-Jones, I. W. Forster,* and *L. J. S. Line* 694

Leaf Development in Swards of White Clover (*Trifolium repens* L.). *R. W. Brougham* 707

The Insect Complex of Sown Pastures in the North Island. II. The General Picture Revealed by Summer Sweep-sampling. *R. A. Cumber* 719

Housing a Guinea Pig Colony. *I. J. Cunningham* 750

Physical and Chemical Changes in the Developing Hop Cone. *H. O. Askew, R. J. Monk,* and *Joyce Watson* 757

Control of *Pieris rapae* by Granulosis Viruses. *J. M. Kelsey* 778

The Leg and the Loin as Indices of the Composition of New Zealand Lamb and Mutton Carcasses. *R. A. Barton* and *A. H. Kirton* 783

Damage in Ryegrasses by *Hyperodes griseus* Hust. *J. M. Kelsey* 790

The Effect of Magnesium Topdressings on the Growth of Young Apple Trees. *H. O. Askew* and *E. Chittenden* 796

Fungal Leaf-spot Diseases of Cocksfoot (*Dactylis glomerata* L.) in the Manawatu. II. Purple Leaf-spot caused by *Stagonospora maculata* (Grove) Sprague, and Leaf Fleck Caused by *Mastigosporium rubricosum* (Dearn. et Barth.) Sprague. *H. T. Wenham* and *G. C. M. Latch* 800

SUBSCRIPTIONS. Subscriptions will be accepted only on advance payment and only for a full calendar year. Price: 30s. per annum; single copy 5s.; post free (surface mail). Published in alternate months, starting February. Subscriptions should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 8018, Wellington, New Zealand. Cheques should be made payable to the Department of Scientific and Industrial Research, and if drawn on banks outside Wellington, must have exchange added. Subscribers are requested to give their name and full postal address.

This journal may also be ordered through The High Commissioner for New Zealand, 415 Strand, London W.C.2, or through any bookseller.

Printed by Coulls Somerville Wilkie Ltd.,
Wellington, New Zealand.

New Zealand Journal of Agricultural Research

VOLUME I No. 6
DECEMBER 1958

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind. however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. 4¼ × 7 in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is 4¼ × 7 in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation, etc., of references, see examples in this issue.

NEW ZEALAND JOURNAL of AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. A. Black.

VOL. 1. DECEMBER 1958 Number 6

BLOAT IN CATTLE

IX. SOME OBSERVATIONS ON THE CULTURE OF THE CATTLE RUMEN CILIATE *Epidinium ecaudatum* Crawley OCCURRING IN QUANTITY IN COWS FED ON RED CLOVER (*Trifolium pratense* L.)

By A. E. OXFORD, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 12 June 1958*)

Summary

In spring and autumn, but not so markedly in high summer, the ciliate *Epidinium ecaudatum* was found to be the predominant oligotrich in the rumens of two cows fed exclusively on red clover. A method has been devised whereby massive suspensions of this ciliate, in the actively motile state and virtually free from holotrich ciliates and bacteria, can be separated from rumen contents *in vitro* in 1 day. This involves the use of a buffered medium, simulating rumen liquid, which contains 0.5% mannose to kill the holotrichs and penicillin and/or neomycin (*ca.* 0.003%) to kill bacteria. By judicious feeding with a preparation of red-clover starch grains containing a little green plant material, such a culture of *E. ecaudatum* has been kept alive *in vitro* for 8 days in presence of a high concentration of penicillin during which time cell division was observed to take place. A high partial pressure of carbon dioxide gas is essential for continued life and activity of *Epidinium.*

INTRODUCTION

Compared with much cultural work carried out on the rumen holotrich ciliates and certain kinds of rumen oligotrich ciliates belonging to the genera *Entodinium* and *Diplodinium** (for summaries of such work see Hungate 1955, and Oxford 1955) there is nothing hitherto in the literature concerning another widely occurring rumen oligotrich genus, *Epidinium,* save descriptions from fixed and stained rumen contents. Since *Epidinium* has now been found to be the predominating oligotrich genus in the rumens of cows fed on red clover at this Station (see below), it appeared to be worth while, as part of a study of the microbial populations in the rumens of cows liable to bloat, to study representatives of this genus in the living state. The ultimate object

---

* In the present paper all generic names follow Dogiel (1927).

N.Z. J. agric. Res. 1: 809–824.

of all such studies, viz, devising a set of conditions whereby one desired rumen ciliate species unmixed with others can be maintained alive indefinitely *in vitro,* has not yet been achieved. However a method has been discovered whereby clean, massive suspensions of epidinia may be obtained which are virtually free from extraneous bacteria and from representatives of other ciliate genera, particularly holotrichs. Such cultures therefore were suitable for disruption by mild methods in order to liberate intracellular enzymes and other cell components characteristic of the genus *Epidinium.* A description of this method, together with observations on the feeding habits of epidinia, form the subject of this paper; the biochemical studies arising therefrom will be reported in a subsequent paper.

Previous Information Concerning Distribution and Feeding Habits of *Epidinium*

Dogiel (1927) devoted 17 pages to *Epidinium* in his comprehensive monograph on rumen oligotrichs and recognised 11 species and subspecies in the genus. It appears to be more widespread in cattle than in sheep since in 32 specimens of rumen contents from the latter animal obtained by Dogiel in Russia, he recognised only 3 subspecies, whereas 9 subspecies were encountered in cattle rumen contents from the same geographical regions. The present author, in a fairly extensive acquaintance with sheep rumen contents in N.E. Scotland, never encountered the genus at all, although it was usually present in cattle rumen contents from the same districts. The closely related and correspondingly widely distributed genus in sheep is *Ophryoscolex*, which in the present author's experience was common enough in Scottish sheep, but not in cattle. Dogiel also found *Ophryoscolex* to be more common in sheep than in cattle judging by the number of different species claimed in association with the respective ruminants (6 as against 4). The two genera are so similar at first sight morphologically that one often has to count the number of contractile vacuoles (two in *Epidinium;* many more than two in *Ophryoscolex*) to be quite sure which genus one is working with. *Ophryoscolex* was not found either in sheep or in cattle at this Station. It has never been kept alive *in vitro* longer than overnight (Eadie *et al.* 1956) and nothing is known about its natural food.

Epidinium is one of the larger rumen ciliates (average dimensions at widest part are of the order of 150 x 50 x 50 μ). Unlike other rumen oligotrichs it is definitely a "streamlined" organism. When in a state of "nutrition" as distinct from "hunger" it contains, like *Ophryoscolex*, three extensive longitudinal skeletal plates which tend to fuse together towards the anal end of the ciliate (Dogiel 1927). These rigid skeletal plates stain brownish purple with iodine and like the corresponding structural reserve material of *Diplodinium* almost certainly consist of a polysaccharide of the starch-glycogen group. (See Oxford 1955 for a discussion of this question.) Like *Diplodinium* and *Entodinium* also, *Epidinium* has the power of laying down a similar polysaccharide in very small granules in certain regions in its ectoplasmic zone (see below). Like *Ophryoscolex*, it has by no means as tough a cuticle as *Diplodinium* or *Entodinium,* and is fairly easily disrupted by gentle mechanical means, or by surface-active agents, in this respect being intermediate between the last-named genera and the holotrichs.

Dogiel noted that *Epidinium*, unlike *Diplodinium,* did not seem to ingest distinct cellulose fibres but rather "meistens kleine Pflanzenpartikeln". Lubinsky (1957) has recently stated that *Epidinium,* like *Entodinium,* feeds upon plant starch, but his observations were based upon fixed and stained, not upon living, material. If true, the population level of *Epidinium* in the rumen may well depend upon the starch content of the forage eaten by the animal. For example, grass contains very little starch indeed before the flowering stage is reached (Percival 1952, Bailey 1958) whereas clover leaves but not stalks at certain times of day, depending on temperature and amount of sunlight, can contain relatively much starch. This particular kind of plant starch is in the form of unusually small grains about 1 μ in diameter (Bailey 1958) which are actually smaller than the individual intracellular storage starch grains produced by certain rumen ciliates, mentioned above. The holotrichs, for example, store amylopectin as oval granules, 3 μ x 2 μ in size (Oxford 1955). Actually both Dogiel's and Lubinsky's observations have been confirmed in the present study.

METHODS

Animals and Their Rations

Rumen-fistulated cows Nos 1, 293, and 294, were chiefly used. They were stall-fed twice daily exclusively on freshly harvested red clover (*Trifolium pratense*), with the morning feed at about 8 a.m., save for certain weekends when they were turned out to a mixed clover/ryegrass pasture. During these periods they were sometimes in company with sheep and therefore liable to acquire, and in fact apparently did so acquire, representatives of the sheep rumen ciliates. Cows 293 and 294 were identical Jersey twins and the latter, but not the former, was dosed with penicillin orally upon occasion (noted below) in connection with experiments on bloat. On one occasion the rumen contents of a fourth cow (No. 2) were examined after the animal had been dosed with paraffin. For purposes of comparison, rumen contents of two sheep that had been freely grazing for some time on a clover/ryegrass pasture were also examined.

Preparation of Food for Ciliates in vitro

Clover leaves were gathered in the early afternoon of a sunny day and the procedure of Bailey (1958), described under "Sampling and processing of the plant tissue", was then carried out as far as the centrifuged solid stage, quartz sand being omitted. Instead of being freeze-dried, the deposit was resuspended in a large volume of distilled water and then distributed in a number of filled 50 ml boiling tubes which were left to stand for several hours at room temperature. Green plant material was first deposited and as soon as this deposit began to be overlaid with a white starch layer, the supernatant was carefully decanted and centrifuged until the final supernatant was clear. The centrifuged solid, drained as far as possible free from water, was dried in a vacuum desiccator over concentrated H_2SO_4 to yield white starch-like lumps covered on top by a very thin greenish layer ("CSc = clover starch plus a little chlorophyll"). The first deposits of green plant material were also combined, centrifuged and dried similarly to yield flakes of green plant material (GPM) more or less free from starch.

Buffers for Maintenance of Living Ciliates in vitro

Preliminary experiments showed that it was necessary to have both bicarbonate and a reducing agent present in order to keep the rumen oligotrich ciliates from these cattle alive overnight *in vitro* (cf. Sugden 1953). The following acetate-phosphate-bicarbonate buffer (APB) based on that used by Sugden and Oxford (1952) for the cultivation of rumen holotrichs was therefore used for most of the experiments described below.

Composition of APB Buffer. The invariable constituents of the buffer were (g/1): NaCl, 5; $CH_3COONa, 3H_2O$, 1.8; $NaHCO_3$, 1.0; KH_2PO_4, 1.0; K_2HPO_4, 0.2; $MgSO_4, 7H_2O$, 0.1; $(CH_3COO)_2Ca$, 0.05; $Na_2S, 9H_2O$, 0.8. Distilled water was used and the initial pH was 6.8–7.0. The complete buffer, not always employed, contained also an antibiotic, usually potassium benzylpenicillin (0.03 g/1). This buffer is 0.14M with respect to volatile fatty acid, i.e. about equivalent to rumen liquid with its maximum VFA content. It will be noted that of the buffering agents in APB, phosphate, bicarbonate, and sulphide are all effective in the same pH range, viz 5.5–7.5; below pH 5.5 acetate (pK_a 4.7) alone supplies the buffering power. The initial penicillin concentration in the complete APB is high (50 units/ml). Hence those rumen ciliates unaffected by penicillin will have to compete for carbohydrate substrates with only a small minority of rumen bacteria, namely those highly resistant to penicillin. In the case of plant starch grains the bacterial competition is even less effective than might be supposed since not only may the ciliates swallow the grains whole, thus removing them from bacterial attack, but amylolytic bacteria themselves, as is well known, are far less effective against starch in the form of native grains than against starch when heat-dispersed in a paste. Other antibiotics were also tried at the same concentration in place of penicillin, viz streptomycin sulphate, zinc bacitracin and neomycin sulphate. Of these, only neomycin gave satisfactory results, streptomycin being very toxic to the oligotrich ciliates whereas bacitracin, although not harming the ciliates, did not appear to be sufficiently bactericidal at the level used. As noted below, APB buffer containing both penicillin (0.004%) and neomycin sulphate (0.003%) proved most satisfactory of all for obtaining bacteria-free suspensions of ciliates for intracellular enzyme studies.

Before use the buffer solution was heated to 38°c in an almost full 1 l. conical flask which was then closed with a rubber bung and placed in the incubator maintained at that temperature. Every few hours, or after overnight standing, the buffer was very briefly gassed with CO_2 from a cylinder in order to maintain saturation with respect to this gas. It is best to make up fresh buffer each day. If any is left from the previous day and is required for use immediately, a further 30 mg of K benzylpenicillin per litre should be added before gassing with CO_2.

Composition of Na-acetate (NA) Buffer. Obviously APB is unsuitable if VFA production by rumen ciliates is under investigation, when a buffer containing no VFA salts is advantageous. The following recipe was used on one occasion: solution I (KH_2PO_4, 1.75g; $MgSO_4$, $7H_2O$, 0.1 g: H_2O, 100 ml) was added slowly with stirring to solution II (NaCl, 5 g; $NaHCO_3$, 1.2 g; H_2O, 600 ml); solutions of Na_2S, $9H_2O$ (0.8 g) in water (200 ml) and K benzylpenicillin (0.03 g) in water (100 ml) were finally added and the whole mixed and heated to 38°c. Buffer NA was found to be much less suitable than APB for long-continued existence of epidinia.

Rapid Method for Obtaining Washed Suspensions of Ciliates

The following method, based on Heald, Oxford, and Sugden (1952), gave best yields when applied to rumen contents removed 4–6 hours after the morning feed of red clover. A 1-litre cylindrical separating funnel with a very short stem, which had previously stood for a while in the incubator, was almost half filled with muslin-strained, well mixed, freshly obtained rumen contents; an equal volume of buffer APB (without penicillin) was then added and the contents mixed by inverting the closed funnel several times. It may be necessary to release gas from time to time during this operation. The unstoppered funnel was then allowed to stand in the incubator for 30 min, when separation into 3 clearly defined zones usually took place. These were a layer of thick green scum, above a column of pale-green turbid liquid, in turn above a greyish-green layer of ciliate protozoa. This bottom layer was drawn off in 3 or 4 successive portions, each into 50 ml of APB (still without penicillin) in a separate boiling tube. A further small yield of ciliates could be obtained by running off the newly formed bottom layer 15 min later. After thorough mixing, the boiling tubes were allowed to stand for 10–15 min, when the supernatant above the bottom protozoan layer was carefully poured off and replaced by fresh APB buffer, this time and subsequently with penicillin dissolved in it. By fourfold or fivefold repetition of this process of washing by decantation at 38°c, a stage was reached when the protozoa fell rapidly to the bottom after mixing the contents of the tube, leaving an absolutely clear and colourless supernatant. At this stage their gas production was very obvious. They were then ready for conversion into a "culture" as described below.

Cultivation of Ciliates in vitro

The method of Sugden and Oxford (1952) employing 50– and 100–ml Erlenmeyer flasks was used with certain modifications, the reasons for which are given at the end of the description of the process which follows. The flasks, each containing a layer of protozoa just covering the bottom, were always kept nearly filled with the complete APB buffer and tightly closed by rubber bungs. Owing to copious gas evolution it was necessary during the first day to tie these bungs firmly down. Usually the protozoa derived from 200 ml of original rumen contents sufficed for a 50-ml culture, to which, immediately upon constituting, was added mannose (0.25 g, L. Light and Co.) dissolved in a little buffer. If microscopic examination (see below) had revealed that the oligotrich ciliates as harvested were mostly well filled with green material, no extra nutriment was added at this stage. Otherwise a small amount of CSc (one drop of a thick, smooth suspension in APB) was also added before closing the flask. During the second day the buffer was changed at least 5 times, by careful decantation of the supernatant with the minimum of disturbance of the bottom layer followed by immediate replacement by fresh buffer and subsequent mixing of the whole contents of the flask. A feed of CSc was sometimes given at the first, and a little GPM at the third change of buffer. Matters were so arranged that starch always preponderated over green plant material before closing the flask immediately prior to overnight incubation. The culture may be very briefly gassed with CO_2 after each addition of nutriment with possible advantage. It may be assumed that the concentration of mannose in the culture is very small at the end of the second working day; this is when the epidinia can be profitably worked up for their intracellular enzymes. If however it is desired to keep the culture alive as long as possible, the procedure of the second day is continued as long as is practicable with progressively longer intervals between the buffer changes and the feeding with CSc.

In culturing the rumen holotrichs, Sugden and Oxford (1952) used rubber bungs fitted with straight tubes bearing Bunsen valves. This technique is inadequate for epidinia since CO_2 gas is an important nutrient or stimulant for them and hence cannot be allowed to escape from the culture flask. The holotrichs produce much fermentation CO_2 endogenously (Heald and Oxford 1953, Gutierrez 1955) hence the need for retention of CO_2 in the culture flask is not so important with them. Endogenous activity of the oligotrichs is in general much less obvious than with the holotrichs. Although CO_2 gas may no doubt eventually be liberated by the action of lactic acid (a probable product of endogenous fermentation of storage polysaccharide) upon bicarbonate in the buffer, this is at best not an instantaneous process since it proceeds through carbonic acid in equilibrium with dissolved CO_2 (Roughton and Clark 1951). Hence the need, as previously stated, for gassing the APB buffer immediately before use in order to maintain a high pCO_2 in the liquid phase.

The purpose of adding mannose is to kill and disintegrate the holotrich ciliates (Sugden and Oxford 1955). This is almost invariably achieved on overnight incubation. If a few holotrichs were alive next morning, the mannose treatment was repeated for a few hours in order to kill them. Mannose appeared not to be toxic to the oligotrichs in the slightest degree. Without it, the holotrichs in the culture, often present initially in great numbers, would remain alive and active for 2–3 days even if no further nutriment was supplied. Mannose can of course be added later in the life of the culture, and in one way it is definitely advantageous to keep the holotrichs in the culture for 1 day at least since they are copious producers of CO_2 and hydrogen. The drawback to this procedure is that the removal of the mannose must be

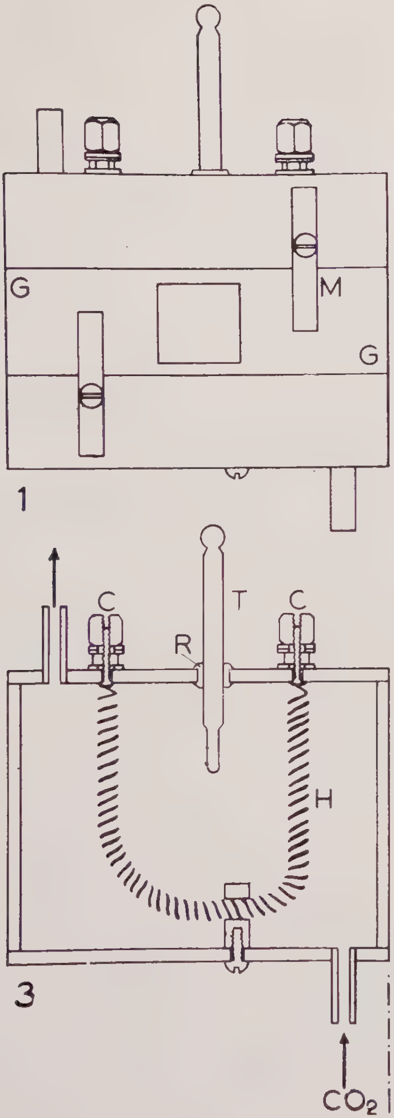


Fig. 1.—Diagram of perspex warm-stage box for studying protozoan activity at 38° c in CO_2 stream.

Dimensions: Overall: 3⅝ x 2¾ x ¾ in.; thicknesses: top ¼ in.; rest ⅛ in.

1: Plan. 2: Side elevation. 3: Horizontal cross section.

CC: Connections to Variac 230v. transformer.

GG: Groove, 1 in. wide 1/12 in. deep, with central hole ¾ in. square, cut in top to take inverted 3 x 1 in. cavity slide with cover slip.

MM: Movable perspex clips for holding slide in place.

H: Heating coil of nichrome wire, resistance 3.6 ohms.

R: Rubber seal holding thermometer in place.

T: Short-stem thermometer, 0–50° c.

carried out in a late stage in the life of the culture when the living oligotrichs may not be as active as they are after incubation for 18 h only.

Microscopic Examination of Active Ciliates Over Long Periods

The precautions of Sugden and Oxford (1952) and Eadie, Mann, and Oxford (1956) were adhered to. These mainly concern the necessity for excluding air and for not allowing the living protozoa to cool below 30°c before a rapid, low-power microscopic examination is carried out. For more leisurely studies of motile ciliates over long periods of time, a special electrically-heated gas-tight shallow box, made of Perspex was designed to serve as a warm stage. It was made by Mr T. O'Donnell in the workshop at this Station. This box (Fig. 1) could take an inverted cavity slide with cover slip, and by means of a variable current its contents could be kept at any desired temperature. A stream of CO_2, or any other gas could be passed through it. It rested on the ordinary movable microscope stage and when the condenser was racked up as far as possible, could be used in conjunction with the $\frac{2}{3}$ in. objective. In order to gauge the correct colour of the protozoan contents it was best used with daylight illumination.

Examination of Protozoan Cultures for Antibiotic-resistant Bacteria

Since, apart from protein and nucleic acids, the main organic products of the action of epidinia on carbohydrates and amino-acids were expected to be lactic and other α-hydroxyacids and storage "starch", attention was paid particularly to bacteria capable of attacking these substrates in the presence of penicillin. Several drops of the final APB washing, used to remove mannose on the second day, were immediately transferred to the following media which were subsequently incubated anaerobically for 2 days at 38°c.

(a) tubes of 1% glucose and 1% sucrose –Bactothioglycollate sloppy agar, some containing 0.1 vol. of the unused APB containing penicillin or other antibiotic;
(b) tubes of lactate sloppy agar according to the recipe of Elsden *et al.* (1956), also with and without penicillin;
(c) 1% glucose—and 1% sucrose –Bacto Brewer's anaerobic agar deeps containing also a little sodium bicarbonate. Growth in (a) was plated on to starch nutrient agar, which was then incubated similarly for detection of amylolytic bacteria as judged by production of clear zones revealed on flooding the plate with dilute Lugol's iodine. Growth in (b), if accompanied by gas, was taken to indicate the presence of lactate-decomposing bacteria. Medium (c) was particularly useful for revealing bacterial growth spreading from individual ciliates embedded in the agar.

RESULTS

Species of Ciliate Protozoa Found in Cattle and Sheep Rumens

Cows No. 1 and 293, in September 1957 when maintained on ryegrass alone, had a considerable population of large oligotrichs together with the usual holotrich mixture (isotrichs and dasytrichs) in their rumens. The oligotrichs were mostly of two kinds only: viz representatives of the genus *Epidinium* and of the subgenus *Eudiplodinium*. The latter name is used, in the sense of Dogiel (1927), to refer to oligotrichs, irrespective of size, which have two ciliary zones roughly in alignment, only two contractile vacuoles and one or two (not more) rather slender skeletal plates. Entodinia were comparatively rare; one small and very motile representative (*Entodinium simplex* ?) being observed. It was noted that in rumen contents of cows that had been starved overnight the majority of the eudiplodinia contained ingested green plant material, whereas the epidinia usually contained none. On the other hand, if the examination of rumen contents was made some 2-5 h after the morning feed, most representatives of this last genus were also seen to be well filled with green plant material. It was concluded that *Epidinium* probably metabolised ingested food material rather faster than *Eudiplodinium*.

For purposes of comparison, the rumen contents of two sheep grazing on a mixed grass and clover sward were examined in December 1957. Each had a much greater variety of oligotrichs, including a higher proportion of entodinia of several species, than cows 293 and 294. Epidinia were however present. The ciliate yields per litre of rumen contents, obtained by the separating funnel method (see above), seemed much less from the sheep than from the cows.

Variations in Population of Epidinium

Cows 293 and 294 were fed on red clover from October 1957 to May 1958. Although their ciliate populations consisted of the same mixture of species at all times, whether on ryegrass or clover, there could be little doubt that *Epidinium* soon became the predominant oligotrich on the latter ration, so much so that in the months of November, December, and January, the ciliate suspension prepared by the separating funnel method described above was to all intents and purposes a mixture of epidinia and holotrichs only. Towards the latter end of February and during March however, the yields of ciliates harvested by the standard method became much smaller, in fact were inappreciable from cow 294 on 6 and 12 March 1958. The relative proportion of epidinia to eudiplodinia and entodinia was also much smaller. *Eudiplodinium neglectum* became especially prominent. In April and May however, the picture gradually reverted to the normal for the spring months and once again good yields of epidinia plus holotrichs were obtained, especially in the middle of May. Towards the end of May, when clover became scarce and hay was fed in addition, the preparations of epidinia became extensively contaminated with a large squat oligotrich, probably *Ostracodinium*.

The yields of epidinia from cows 293 and 294 were at all times comparable, at best *ca.* 3 g wet weight, after centrifuging, per litre of rumen contents even though the latter animal was dosed with penicillin (100,000 units) several times during October-December 1957. There seemed to be no appreciable diminution in ciliate yield from cow 294 some 4–5 h after feeding on the day after penicillin treatment.

Similarly, the dosing of cow 2, on 7 November 1957, with paraffin oil (50 ml) to prevent excessive foaming of rumen contents and subsequent bloat seemed to have had little effect on the ciliate population. Epidinia were also the predominant oligotrichs in cow 2.

Behaviour During Early Stages of Culture

When *Epidinium* contained ingested green plant material it also stained fairly evenly for starch with Lugol's iodine. On the other hand, the living but empty-looking ciliates surviving after the overnight mannose treatment without added CSc or GPM contained little if any green plant material and only their skeletal plates were stained by iodine. These skeletal structures became less prominent if starvation was continued for a further 8 hours. The difference in colour between the green ciliate layer as harvested and the dirty white bottom deposit of ciliates after 1 day's starvation was striking. It was therefore inferred that clover-starch grains, as well as chloroplasts, were the chief plant constituents ingested and digested by epidinia, as stated by Lubinsky (1957), and were converted by the ciliate into its own storage polysaccharide which in turn was used up during periods of starvation. Freshly isolated epidinia from the rumen never contained cellulose fibres inside themselves. It is clear that this ciliate, or intracellular bacteria in association with it, must be able to decompose chlorophyll. The loss of green colour could hardly be ascribed to extra-protozoan bacterial action, since the penicillin—or neomycin—containing supernatants were always reasonably, sometimes completely, clear and did not appear to be more than lightly contaminated with bacteria (see however the section below on bacteria associated with epidinia in antibiotic-containing cultures). A feature not listed by Dogiel was the occasional occurrence, some hours after the culture had been fed with CSc, of protozoan storage polysaccharide, staining purple with iodine, in the form of minute granules in the tail and 'rump' of the ciliate, definitely not a part of a skeletal plate. Uni- and multi-tailed representatives of the genus *Epidinium* occurred in all three bovine rumens, the first-named preponderating. This variety corresponded closely to Dogiel's *E. ecaudatum* forma *caudatum*. The tailless variety *E. ecaudatum ecaudatum* was very rarely observed. The unitailed variety seemed to be the hardiest *in vitro*.

Examination in a CO_2 Atmosphere on a Warm Stage Over Long Periods

Confirmation of the power of epidinia to ingest green plant material and clover-starch grains was obtained by watching a slide culture intermittently over several hours. To this end a drop or two of a 1-day, holotrich-free suspension, which had not received any CSc before overnight incubation, were transferred to fill the depression in a warm cavity slide already containing a little CSc and GPM in suspension. The cover slip was then put on, the slide inverted and placed in position in the warm box at 38°c on the microscope stage. It was advantageous to have a few drops of water on the floor of the box before starting the slow CO_2 stream. The ciliates, which although empty-looking, soon became very actively motile, began to collect in a small area near the edge of the depression (see below for probable explanation of this phenomenon) and after about 30 min it was evident that a few epidinia had taken up green plant material. After 60 min the green-looking organisms formed an appreciable proportion of the living population. Some individuals were also seen which had ingested clover-starch granules without accompanying green material. After 3 hours the trend was even more noticeable, and when the whole preparation was stained with Lugol's iodine, numerous individual ciliates filled with iodophilic material could easily be picked out from others not so filled. At the beginning of the incubation there were hardly any such iodophilic individuals since the culture had received no food after it had been constituted the day before.

Need of Epidinium *for CO_2 Gas*

The rumen ciliates are anaerobic organisms and it has hitherto been thought that it is most important to keep oxygen out of their cultures. The following observations show that the provision of CO_2 gas is even more important to the well being of epidinia than the total exclusion of air. Appproximately 150 ml of a 1-day-old holotrich-free culture of *Epidinium* was fed CSc and then after a few hours' incubation was well mixed and divided into three equal parts, one of which filled a 50-ml conical flask fitted with a rubber bung, and the others only two-thirds filled rather larger conical flasks (75 ml) loosely plugged with cotton wool. The last two cultures had therefore a much greater surface/volume ratio than the first. One plugged flask was incubated overnight in a CO_2-filled tightly closed metal anaerobic jar of capacity 6 litres; the second plugged flask, like the closed flask, was incubated in air. Next morning there seemed little to choose between the motility and storage-starch content of ciliates in the closed flask, and the CO_2 cultures. Both were in a healthy state whereas the culture incubated with access of air was quite moribund. The superiority of the closed flask conditions became evident however when, after refeeding, the cultures were incubated for a further day. The CO_2 had not been freed from traces of O_2.

What may be regarded as a confirmation of the need of epidinia for a high pCO_2 is derived from the observations made on the cavity-slide culture in the warm box noted above. The aggregation of the motile ciliates into a small compact mass near one portion of the edge of the cover slip, which just covered the cavity, might well be because the access of CO_2 to the liquid was easiest at that point owing to an inequality in the slide or the cover slip. If an ordinary slide with cover slip was used under the same conditions, the ciliates soon ceased to be motile under the central portion of the cover slip at a time when they were still motile at the edge and in the liquid expressed beyond the edge of the cover slip. A nearly moribund bulk liquid culture containing the usual concentration of HCO_3 could sometimes be noticeably revived merely by a very brief gassing with CO_2 at 38°c. This, with the other evidence, show that CO_2 gas rather than the bicarbonate ion is the active agent in keeping epidinia motile.

Observations on Long-lived Epidinium *Cultures*

By judicious alternate feeding with GPM and CSc, together with a twice daily changing of the buffer, several cultures were kept alive for 4 days or more, the best lasting for 8 days. Dividing forms were never seen till the third or fourth day. Even so there was a progressive diminution in numbers of living ciliates throughout the life of the culture even allowing for losses incidental to sampling and buffer changing. Towards the end, the few surviving epidinia, although actively motile, were inclined to be considerably larger than at the beginning, in fact closely corresponded to a one-tailed form of the *Epidinium ecaudatum gigas* of Dogiel. His *E. ecaudatum bulbiferum* also was in evidence in old cultures. It is absolutely essential that the buffer should always contain penicillin. Without the antibiotic, starch-decomposing bacteria soon gain the upper hand with speedy lowering of pH and death of the ciliates. On the other hand, in presence of penicillin it seemed much more deleterious to provide the ciliates with excess of GPM than of clover starch. An attempt to replace GPM by an amino-acid mixture (Difco casamino acids, 0.1%; 1-tryptophan, 0.005% in APB) led to no useful result: the epidinia died overnight. On the other hand the holotrichs tolerated the amino-acids quite well in presence of penicillin.

Owing to limitation of supplies of neomycin it was not possible to determine whether epidinia, fed on CSc, could be kept alive for more than 2 days when both penicillin and neomycin were incorporated in the buffer. At the end of this period the ciliates were certainly not less active, and the supernatant was undoubtedly freer from bacteria (see next section) than when penicillin or neomycin alone was employed.

It is probable that *Epidinium*, like the rumen holotrich genus *Isotricha*, requires intact starch grains for food and cannot utilise starch in the form of paste (cf Howard 1957). When clover-starch grains were burst by heating in water at 100°c they no longer served to keep a culture of epidinia alive overnight in an active state. The few organisms that did remain alive had only poorly developed skeletal plates. Nevertheless soluble starch had disappeared from the medium, probably owing to the action of an amylase liberated by the autolysis of moribund epidinia, of which there were great numbers after the overnight incubation.

When fed potato starch in place of clover starch, epidinia were able to ingest only the smallest grains among the mixture, leaving the bulk of the moderately large-sized grains to be ingested by other oligotrichs. The culture was maintained for 7 days on clover GPM and potato starch.

Bacteria Associated with Epidinia in Presence of Antibiotics

Although penicillin acts best against Gram-positive bacteria and neomycin against Gram-negative bacteria, nevertheless the same lactate-decomposing Gram-negative coccus was isolated from APB washings containing either antibiotic on the second day of the starved protozoan culture. This anaerobic organism was intermediate in size between the known rumen Gram-negative cocci *Veillonella gazogenes* (Johns 1951) and the Elsden coccus LC (Elsden *et al.* 1956) and is apparently different from both. It was very penicillin-resistant, and could easily be isolated directly from the rumen contents of these clover-fed cows by use of Elsden's lactate medium containing penicillin (2.5 units/ml). A study of its properties is now being made in this laboratory. Like the Elsden coccus LC, it also ferments glucose and possibly other sugars, but not starch. No starch-fermenting bacteria were in fact isolated from these antibiotic-containing washings. No viable bacteria were isolated at all, in 3 attempts, from similar washings containing both antibiotics. The corresponding washed protozoa however, when transferred to agar medium (c), produced some gas-forming bacterial colonies in 2 days incubation from sucrose as carbohydrate source.

Metabolic Products of Epidinia

On account of bacterial contamination in the metabolic liquid, little can be said at the moment concerning the soluble metabolic products from starch fermentation by epidinia in NA buffer containing penicillin, save that volatile fatty acids were not formed on overnight incubation. Actually the VFA level after incubation (0.4 millimoles/100 ml) was less than at the beginning (0.9 millimoles/100 ml). In this experiment the epidinia were harvested and treated throughout by use of NA buffer, and a small minority of the ciliates were still alive at the end of the experiment.

Epidinia freed from holotrichs do not seem to be able to utilise either glucose or xylose, supplied in 0.1% concentration in APB, to produce iodophilic skeletal plate storage polysaccharide on overnight incubation. In such experiments lactic acid could be detected in the metabolic liquid at the end, but there was no proof that this lactic acid did not arise by bacterial action, or merely by the endogenous fermentation of *Epidinium* storage polysaccharide. Very little monose seemed to be used up in these incubations.

DISCUSSION

The difficulty in biochemical work with the rumen oligotrich ciliates has hitherto been the almost invariable presence, in their suspensions upon first isolation from rumen contents, of the metabolically more active rumen holotrichs. Short of removal of holotrichs by selective defaunation of the ruminant animal by emptying the rumen and pasteurising its contents before returning (Eadie and Oxford 1957) which method is hardly likely to succeed as well with bovines as with the smaller sheep, it is believed that the above-described *in vitro* method, in which mannose is employed selectively to kill the holotrichs without harm to the oligotrichs, is the best available to date for obtaining the latter in 'pure' suspension. Antibiotics are of no use for this purpose since the oligotrichs are in general more susceptible to them than the holotrichs. Streptomycin, for example, is non-toxic to the holotrichs (Heald and Oxford 1953) but distinctly toxic to oligotrichs of at least three genera, viz *Eudiplodinium, Entodinium* (Sugden 1953), and *Epidinium,* as found in this present work. The quick separation of one oligotrich genus from another in mixed suspension is still difficult however; it is only when one genus dominates all others in the rumen, as in the present study with an exclusively red-clover ration, that worthwhile biochemical work upon its enzymes becomes possible in the present state of our techniques.

The present study, admittedly with two cows only, suggests that the population level of *Epidinium* in the rumen, even when the animals are on an exclusively red-clover ration, is far from constant, being much higher in spring and autumn than in high summer. Whether this variation can be correlated with any change in the average chemical composition of the clover, particularly with regard to starch, remains to be investigated. Certainly *Epidinium* seems to feed upon the small-grained red-clover starch and also upon the specifically green plant material, i.e. presumably chloroplasts, whole or damaged. The green material is also ingested with avidity by other oligotrich genera, particularly *Eudiplodinium;* not so, however, the red-clover starch grains. Hitherto it has been thought that *Entodinium* is the starch-eating rumen oligotrich genus *par excellence* (cf. van der Wath and Myburgh 1941), but most observations upon this genus were obtained when the host animal was fed a grain ration containing starch of much larger granule size than red-clover starch. It may be that *Entodinium* is not fitted to swallow starch grains below a certain limiting size. Certainly the population of entodinia in the rumens of the 3 cows fed red clover in this study was surprisingly small, in view of the not inappreciable starch content of red clover, in the daytime at least. In view of the recent population study with adequate statistical control carried out by Boyne *et al.* (1957), in which surprisingly large daily variations occurred in the count of both large and small ciliates in the rumen of a sheep fed a constant hay and concentrate ration over a period of 19 days, it is admittedly a little difficult to see how the activity of epidinia in the rumen can be satisfactorily correlated with variation in the ration fed to the host animal, if all that is done is merely to count rumen populations. Perhaps a differential count multiplied by the mean content of iodophilic polysaccharide inside the ciliate might give a more satisfactory indication of *in vivo* protozoan activity due to a given genus. This is a matter for future research.

In obtaining suspensions of living epidinia for enzyme studies, it is obviously important to remove virtually all living extracellular bacteria at least, leaving only those (if any) which may live in association with the ciliate in its endoplasm. It appears that the use of a buffer containing both penicillin and neomycin for washing the ciliates has to all intents and purposes achieved this end, and that such a buffer is more suitable than one containing only one of these antibiotics. This is not surprising since penicillin and neomycin are known to be synergistic in their antibacterial action (Heilman 1953). Whether living and propagating *Epidinium* cultures can really be maintained in a bacteriologically sterile state has still to be determined. Bacteria will of course be introduced into the culture with the dried clover starch. There seems also to be an antibiotic-resistant lactate-decomposing Gram-negative coccus in the rumens of cows fed on red clover which is difficult to eradicate completely from ciliate suspensions. On the other hand, a culture of *Epidinium* has been kept alive *in vitro* for 8 days in the presence of a high concentration of penicillin, so it would seem that this particular rumen coccus is well tolerated by this particular ciliate. In general rumen ciliates and bacteria not of rumen origin do not live in harmony *in vitro*, the bacteria soon getting the upper hand; hence the need for antibiotics in most cultural work with rumen ciliates. Unlike streptomycin, neither penicillin nor neomycin seems to harm *Epidinium* in the least.

The fact that epidinia need CO_2 gas for full activity has important implications for *in vivo* studies because the rumen gases are not always chiefly CO_2 and methane. After overnight starvation the chief rumen gas is nitrogen and the partial pressure of CO_2 can be quite small (Brody 1945). This means that epidinia, and perhaps other oligotrich ciliates, will not then become fully active immediately after the introduction of fresh food into the rumen. There might well be a time lag during which bacterial fermentation has to produce enough CO_2 gas to saturate the rumen liquid.

ACKNOWLEDGEMENTS

The antibiotics used in this investigation were kindly supplied by the Development Department of Glaxo Laboratories (N.Z.) Ltd.

It is a pleasure to acknowledge the invaluable collaboration of Mr Campbell Reid and Mr P. Vlieg of the Physiology Section of this Division, who maintained the experimental animals and supplied rumen contents as required. Mr Vlieg is also responsible for the diagram. Thanks are also due to Mr T. O'Donnell for designing and making the warm-stage box and to Miss C. E. Arbon for technical assistance.

REFERENCES

BAILEY, R. W. 1958: Carbohydrates in Pasture Species. I. The Starch Content of Clovers and Rye-grass. *J. Sci. Fd. Agric.*

BOYNE, A. W.; EADIE, J. M.; RAITT, K. 1957: The Development and Testing of a Method of Counting Rumen Ciliate Protozoa. *J. gen. Microbiol. 17:* 414.

BRODY, S. 1945: "Bioenergetics and Growth." Reinhold, New York.

DOGIEL, V. A. 1927: Monographie der Familie Ophryoscolecidae. *Arch. Protistenk. 59:* 1.

EADIE, J. M.; OXFORD, A. E. 1957: A Simple and Safe Procedure for Removal of Holotrich Ciliates from the Rumen of an Adult Fistulated Sheep. *Nature 179:* 485.

EADIE, J. M.; MANN, S. O.; OXFORD, A. E. 1956: A Survey of Physically Active Organic Infusoricidal Compounds and their Soluble Derivatives with Special Reference to their Action on the Rumen Microbial System. *J. gen. Microbiol. 14:* 122.

ELSDEN, S. R.; VOLCANI, B. E.; GILCHRIST, F. M. C.; LEWIS, D. 1956: Properties of a Fatty Acid Forming Organism Isolated from the Rumen of Sheep. *J. Bact. 72:* 681.

GUTIERREZ, J. 1955: Experiments on the Culture and Physiology of Holotrichs from the Bovine Rumen. *Biochem. J. 60:* 516.

HEALD, P. J.; OXFORD, A. E. 1953: Fermentation of Soluble Sugars by Anaerobic Holotrich Ciliate Protozoa of the Genera *Isotricha* and *Dasytricha*. *Biochem. J. 53:* 506.

HEALD, P. J.; OXFORD, A. E.; SUGDEN, B. 1952: A Convenient Method for Preparing Massive Suspensions of Virtually Bacteria-free Ciliate Protozoa of the Genera *Isotricha* and *Dasytricha* for Manometric Studies. *Nature 169:* 1055.

HEILMAN, F. R. 1953: Antibiotics. *Ann. Rev. Microbiol. 7:* 225.

HOWARD, B. H. 1957: Biochemical Studies of Individual Genera of Rumen Holotrich Protozoa. *Biochem. J. 67:* 18P.

HUNGATE, R. E. 1955: The Ciliates of the Rumen. *In* "Biochemistry and Physiology of Protozoa." Vol. 2. Ed. Hutner, S. H. and Lwoff, A. Academic Press, New York.

JOHNS, A. T. 1951: Isolation of a Bacterium Producing Propionic Acid from the Rumen of Sheep. *J. gen. Microbiol. 5:* 317.

LUBINSKY, G. 1957: Studies on the Evolution of the Ophryoscolecidae (Ciliata: Oligotricha). III. Phylogeny of the Ophryoscolecidae Based on their Comparative Morphology. *Canad. J. Zool. 35:* 141.

OXFORD, A. E. 1955: The Rumen Ciliate Protozoa: their Chemical Composition, Metabolism, Requirements for Maintenance and Culture, and Physiological Significance for the Host. *Exp. Parasitol. 4:* 569.

PERCIVAL, E. G. V. 1952: The Carbohydrate Constituents of Herbage. *Brit. J. Nutr. 6:* 104.

ROUGHTON, F. J. W.; CLARK, A. M. 1951: Carbonic Anhydrase. *In* "The Enzymes". Vol. I, Part II; Ed. Sumner, J. B. and Myrback, K. Academic Press, New York.

SUGDEN, B. 1953: The Cultivation and Metabolism of Oligotrich Protozoa from the Sheep's Rumen. *J. gen. Microbiol. 9:* 44.

SUGDEN, B.; OXFORD, A. E. 1952: Some Cultural Studies with Holotrich Ciliate Protozoa of the Sheep's Rumen. *J. gen. Microbiol. 7:* 145.

VAN DER WATH, J. G.; MYBURGH, S. J. 1941: Studies on the Alimentary Tract of Merino Sheep in South Africa. VI. The Role of Infusoria in Ruminant Digestion with some Remarks on Ruminant Bacteria. *Onderstepoort J. vet. Res. 17:* 61.

BLOAT IN CATTLE

X. THE CARBOHYDRASES OF THE CATTLE RUMEN CILIATE *Epidinium ecaudatum* Crawley ISOLATED FROM COWS FED ON RED CLOVER (*Trifolium pratense* L).

By R. W. Bailey, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 4 July 1958*)

Summary

Cell-free extracts have been prepared from virtually pure cultures of *Epidinium ecaudatum* Crawley, a protozoon which may be abundant in the rumen of cows feeding on red clover. Water extracts contain an amylase while phosphate buffer extracts contain the same amylase together with a less soluble maltase and occasionally a cellobiase. Invertase and β-glucosidase were absent from the extracts which were also unable to hydrolyse lactose, melibiose, $\alpha\alpha$-trehalose and cellulose.

The amylase has been shown to be an α-amylase with optimum activity in the pH range 5.3–6.5 and in the temperature range 37°–45°c. The final products of the action of the amylase on starch are maltose (major component) and glucose and maltotriose (minor components). Intact clover-starch granules were apparently hydrolysed by the amylase but potato-starch granules were not attacked.

Introduction

Of the protozoa of the rumen only members of the holotrich group have been obtained sufficiently pure for an examination of their intracellular carbohydrases to be made (Howard 1957; Mould and Thomas 1957). The carbohydrases secreted by the oligotrich species do not appear to have been examined, presumably because of the difficulty encountered in obtaining them as pure active cultures. In the previous paper of this series (Oxford 1958) it was shown that under certain feeding conditions (fresh red-clover diet) practically the only oligotrich present in the rumen of cows was *Epidinium ecaudatum* Crawley. A method was devised which provided active cultures of this species that were entirely free from holotrichs and extraneous bacteria. Of particular interest in connection with this oligotrich was the fact that its abundance in the rumen appeared to depend on the presence in the clover leaves of small starch granules (Bailey in press, 1958; Oxford 1958). In contrast to other oligotrichs *Epidinium* readily ingests these granules, and they are apparently necessary for the maintenance of active *in vitro* cultures of the organism. It was decided to amplify these observations by examining the carbohydrases which could be extracted from the pure epidinia cultures.

This paper reports the results obtained in an investigation of the carbohydrases extracted from disrupted epidinia. Some properties of the major carbohydrase present in the extracts (an α-amylase) are also recorded.

Experimental

Epidinium Cultures

Virtually pure cultures of the organism, which had been starved overnight and then well washed (Oxford 1958) were used. The protozoa were compacted by centrifuging at 14,000 g on a Spinco preparative ultracentrifuge.

Preparation of Cell-free Epidinium Extracts

Epidinia (2–4 g, wet wt) were suspended in water (20 ml) and macerated in a Teflon tissue disintegrator for a total time of 30 min in periods of 2–3 min. During the maceration the preparation was kept cooled in ice-water and at intervals a drop of the macerate was removed, stained with iodine to facilitate examination, and inspected under a low-power microscope. A total of 25–30 min maceration gave, by visual inspection, about 90% disintegration of the protozoa. The final suspension was centrifuged for 30 min at 20,000 g and 0–5°c and the supernatant collected with the aid of a hypodermic syringe. The residue of cell debris was reground with water (20 ml) at 0°c for a total time of 10 min and a second extract obtained. On several occasions the water used in the two extractions was replaced with a phosphate buffer (KH_2PO_4 - Na_2HPO_4 0.003M. NaCl 0.02M, $MgSO_4$ 0.001M; pH 7.0). After the two buffer extractions the residue of cell debris was ground again with the same buffer mixture at ten times the strength given above. All of the extracts were centrifuged a second time for 30 min at 26,000 g and 0–5°c to remove any traces of cell material and stored at 2°c under toluene.

Examination of the Extracts for Carbohydrase Activity

The action of the epidinium extracts on various carbohydrates was tested in mixtures containing epidinium extracts (1 ml) and added carbohydrate (10 mg) in acetate buffer (0.05M, pH 6.5, 3 ml). The solutions were examined by paper chromatographic analysis both before and after incubation at 37°c. Mixtures containing sugars were incubated for 4 h and those containing starch fractions or cellulose were incubated for 24 h under toluene. Paper chromatograms were prepared and developed by the usual techniques using a solvent consisting of the top layer of a mixture of ethyl acetate, water, and pyridine (2:2:1 by vol. Jermyn and Isherwood 1949). Sugars were detected on the papers with the acetone-silver nitrate spray of Trevelyan *et al.* (1950).

The presence on the papers, in more than trace amounts, of saccharides of shorter chain length than the substrate sugar or polysaccharide in the solution (e.g. glucose from maltose, maltose from starch) was taken as evidence of the particular carbohydrase activity. Control solutions containing the added carbohydrate but no epidinium extract were run in all cases. Other controls showed that the epidinium extracts themselves contained no free sugars and did not produce any when incubated in the acetate buffer at 37°c for 24 h.

Carbohydrate Substrates

All of the sugars used (B.D.H., or L. Light and Co.) were chromatographically pure. Powdered cellulose (Whatman's chromatography-ashless) was suspended in water and dialysed overnight at 0°c against distilled water before use.

An amylose fraction was isolated from potato starch by the aluminium hydroxide-thymol method of Hobson *et al.* (1951). β (limit)-dextrin was prepared by the action of soya-bean β-amylase on unfractionated potato starch (Hobson *et al.* 1950). Intact clover-starch granules were prepared by the method described by Oxford (1958). Intact potato-starch granules were isolated by macerating freshly peeled sliced potatoes for 3 min in a blendor, squeezing the macerate through cheese-cloth and allowing the starch granules to settle under gravity. The granules were washed several times by resuspension in water until a clean white sediment and a clear supernatant were obtained.

Amylase Preparations

In order to compare epidinium amylase with amylases of known characteristics authentic specimens of β and α amylase were prepared. β-amylase was prepared from soya-bean flour by the method described by Bourne *et al.* (1945). Before use, solutions of the enzyme in acetate buffer (0.05M, pH 6.5) were dialysed against the same buffer (2 1) at 2°C for 24 h. A preparation of salivary α-amylase was obtained by centrifuging freshly collected human saliva for 30 min at 14,000 g to remove mucin, etc. Before use, the supernatant was diluted with water (1/100) to give a suitable concentration of activity.

Measurement of α-Amylase Activity

The method of Hobson and Macpherson (1952) for measuring α-amylase activity, which depends on the loss of iodine staining power, was used with minor modifications. Amylose (87.5 mg) was dissolved by warming in 0.175N NaOH (15 ml), neutralised with N H_2SO_4 and diluted to volume (50 ml). β-dextrin (87.5 mg) was dissolved in water and diluted to 50 ml. Solutions were prepared containing amylose solution (4 ml), enzyme (0.5 ml), and citrate buffer (0.2M, pH 6.5, 2.5 ml) so that the final mixtures contained 1 mg of amylose per ml. In the case of β-dextrin mixtures, twice these volumes were used. Buffer and starch solutions were preheated to the incubation temperature. The solutions were incubated at 37°C (unless stated otherwise) and at intervals portions (amylose solutions 1 ml, β-dextrin solutions 2 ml) were withdrawn and stained with iodine (iodine 1 mg, potassium iodide 10 mg in water 100 ml).

Using the Beckman model DU spectrophotometer the extinction coefficients (E) were measured at 680 mμ for the deep blue iodine-amylose complex and at 560 mμ for the reddish purple iodine-β-dextrin complex. The yellow colour due to unreacted iodine did not interfere at either of these wavelengths. Control solutions containing the starch fraction but no enzyme were also run. The amylase activity of an enzyme mixture, at any given time, was expressed as the percentage change of the extinction value of its iodine complex relative to the extinction value of the control solution. Variations in the above method, e.g. in assessing the effect of pH or temperature on activity, are described below.

RESULTS

Action of Epidinium Extracts on Various Carbohydrates

Both water and phosphate-buffer extracts were tested against sucrose, lactose, melibiose, raffinose, cellobiose, maltose, amygdalin, salicin, α-methyl glucoside, αα-trehalose, powdered cellulose, β-dextrin, and amylose. Consistent activity was obtained only in the maltose, β-dextrin, and amylose solutions. The need for a pure epidinium culture was shown by the fact that the extracts from an early preparation, known to contain a few holotrichs, possessed strong activity against sucrose and the β-glucosides.

Examination of the paper chromatograms indicated the presence of strong amylase activity in both of the water extracts and in the 0.003M phosphate-buffer extracts. The final 0.03M phosphate buffer extract appeared to contain very little amylase activity. Maltase activity was absent from the water extracts, weak in the 0.003M phosphate-buffer extracts and strong in the 0.03M phosphate-buffer extract. Cellobiase activity, absent from the water extracts was, on one occasion, detected in the first, but not in the second and third (0.03M), buffer extract. Because of the lack of further supplies of epidinia it has not been possible to check whether this enzyme is always present.

Nature of the Amylase

As the amylase was the most abundant carbohydrase obtained, its properties and identity were examined in detail. Two major types of amylases are known:—

β-Amylases which cleave alternate α 1 ⟶ 4 glucosidic linkages in the starch and liberate maltose. This type of enzyme can completely degrade amylose but its attack on amylopectin stops at the α 1 ⟶ 6 glucosidic branch linkages. The resulting β or limit-dextrin is unattacked by a β-amylase.

α-Amylases which can degrade amylose, amylopectin, and β-dextrin. With this type of amylase there is a more random attack on the α 1 ⟶ 4 linkages to produce initially short-chain dextrins. Only in the later stages of the degradation is a mixture of glucose, maltose, and maltotriose produced.

Many crude enzyme preparations (e.g. plant extracts) contain both enzymes while those from other sources (e.g. human saliva) yield only one. The simplest means of identifying the amylases in an extract is the method devised by Hobson *et al.* (1950) as used by Hobson and Macpherson (1952) in identifying two rumen bacterial amylases. In this method the activity of the enzyme in standard mixtures containing amylose and in those containing β-dextrin is measured at equal time intervals. The results obtained with each starch fraction, at each time interval, expressed as a percentage change in extinction of the iodine-starch complex are plotted against one another as in Fig. 1. Pure β-amylase, attacking only amylose, gives a curve of type A, while pure α-amylase, which attacks amylose and β-dextrin equally, gives a curve of type B. Mixtures of the two enzymes give curves intermediate between curves A and B. The curves are independent of enzyme concentration.

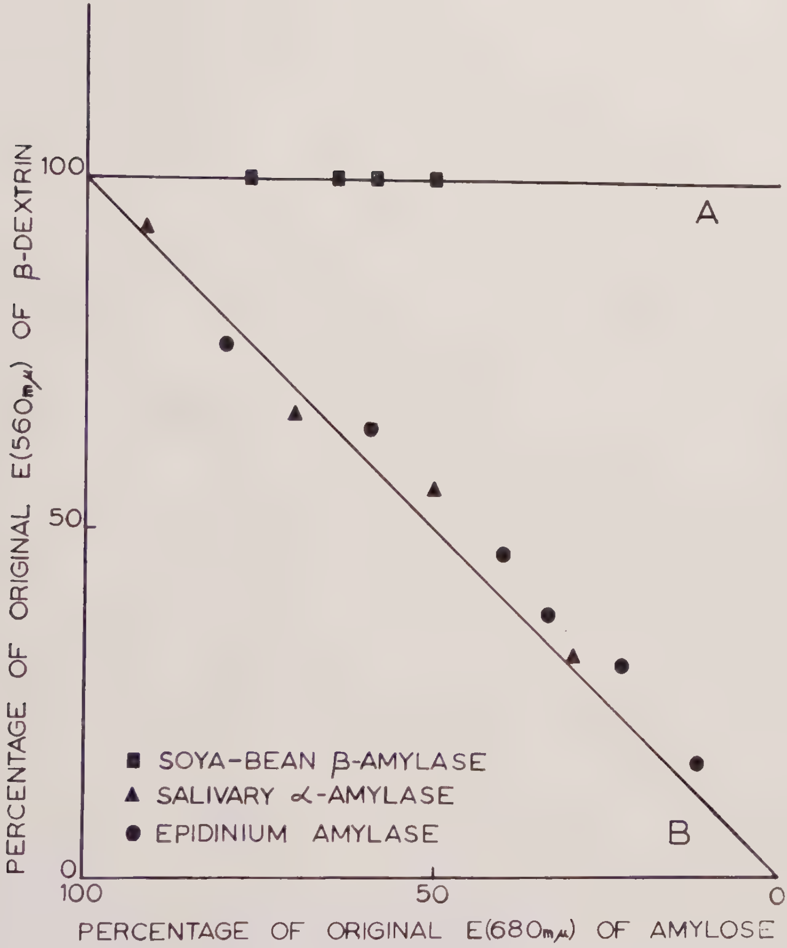


Fig. 1.—The comparative action of amylases of soya-bean, saliva and epidinium on amylose and β-dextrin. A, theoretical curve for β-amylase. B, theoretical curve for α-amylase. All solutions incubated at 37°C and pH 6.5.

Typical results obtained with soya-bean β-amylase and human salivary α-amylase together with the results obtained with the epidinium amylase (aqueous extract) are plotted in Fig. 1. From these results it is evident that the epidinium amylase is a pure α-amylase. This is confirmed by paper chromatographic evidence which showed the production, after 24 h incubation, of the same mixture of oligosaccharides, at about the same concentration, from both amylose and β-dextrin by the epidinium extract.

End Products of α-Amylase Activity

Mixtures containing either amylose or β-dextrin (10 mg) and water extracts of epidinium (1 ml; free from maltase) in acetate buffer

(0.05M, pH 6.5, 3 ml) were incubated for 24 h at 37°C. Paper chromatograms of the incubated digests showed three reducing components, which were chromatographically identical with glucose, maltose, and maltotriose. The glucose and maltotriose components were present in small amounts only, while the maltose component constituted at least 90% of the total sugars on the papers.

Effect of pH on α-Amylase Activity

The effect of pH on the amylase activity of epidinium water extracts was investigated using standard-activity solutions in which the pH of the added citrate buffer ranged from 4.0 to 7.3. After incubation for 30 min at 37°C, amylase activity was measured by the usual iodine-staining technique and the pH of the incubated digests was measured. The results obtained, plotted in Fig. 2, show that the pH optimum was in the range 5.3–6.5.

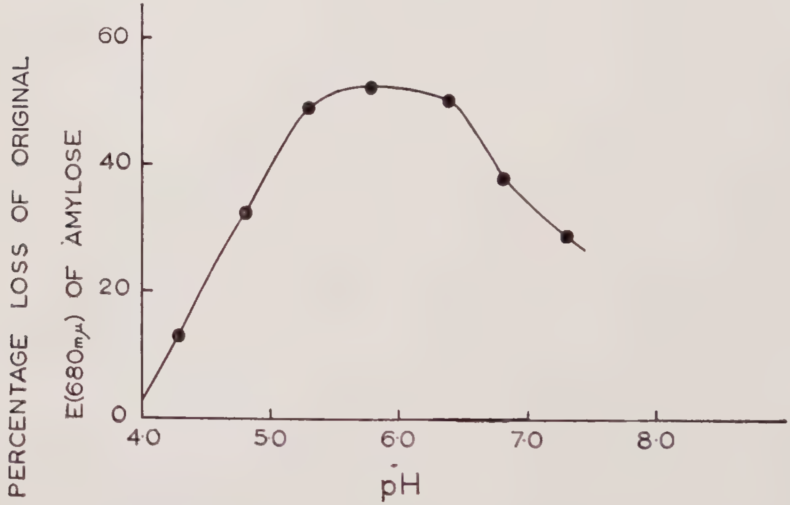


Fig. 2.—Effect of pH on activity of epidinium α-amylase at 37°C. Solutions, containing citrate buffer (2 ml 0.02M), incubated for 20 min.

Effect of Temperature on α-Amylase Activity

Amylase activity was measured as above in standard-activity solutions except that they were incubated for 30 min at different temperatures. The results obtained with a water epidinium extract are plotted in Fig. 3 and show that the maximum activity extends over the temperature range 37°–45°C. Similar results were obtained using the first phosphate-buffer (0.003M) extract of the epidinia.

Action of α-Amylase on Intact Starch Granules

Solutions were prepared containing epidinium extract (first phosphate buffer, 0.003M, extract) and intact clover- or potato-starch

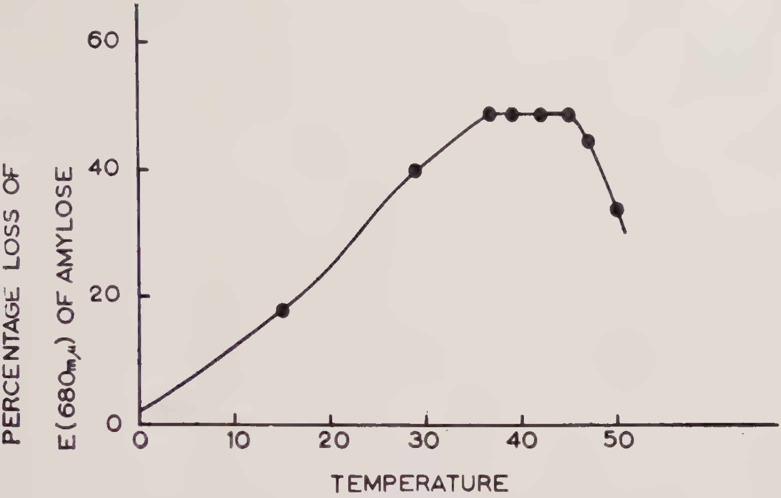


Fig. 3.—Effect of temperature on activity of epidinium α-amylase. Solutions (pH 6.5) incubated for 30 min.

granules (10 mg). Controls containing the starch but no enzyme were run at the same time together with solutions containing dispersed starch (amylose) with and without the epidinium extract. After incubation for 24 h at 37°c under toluene the digests were examined by paper chromatography. Two solutions, those containing the epidinium extract with dispersed starch and with clover-starch granules after incubation showed, on the paper, components corresponding to glucose, maltose, and maltotriose. All of the controls and the potato-starch solutions gave blank chromatograms.

DISCUSSION

Of the two major groups of rumen ciliate protozoa, oligotrichs and holotrichs, the latter are present in the rumen irrespective of diet and appear to be able to utilise a variety of carbohydrates. Two genera (*Dasytricha* and *Isotricha*) have been shown to contain an α-amylase (Mould and Thomas 1957). In addition Howard (1957) reported that extracts of *Dasytricha ruminantium* contained cellobiase, maltase, invertase and a powerful β-glucosidase whilst extracts of *Isotricha* contained both a β-glucosidase and a powerful invertase. Extracts of both organisms were also able to hydrolyse laminaribiose, raffinose, inulin, and bacterial levan but not melibiose, lactose or αα-trehalose.

In contrast to the above results the epidinium extracts contain no invertase or β-glucosidase. The only carbohydrases extracted are an α-amylase, a maltase, and occasionally a cellobiase. Like the holotrichs, the epidinium extracts are without action on melibiose, lactose and αα-trehalose. The holotrich β-glucosidase and invertase are powerful enzymes and contamination of the epidinia cultures with even a few holotrichs could give misleading results. Hence in working with oligotrich enzymes care must be taken to see that the cultures used are truly holotrich-free.

As with other bacterial and animal amylases (Hobson and Macpherson 1952) the epidinium enzyme is an α-amylase. It possesses maximum activity over the pH range 5.3–6.5 and the temperature range 37°–45°C. Generally the rumen contents of cows feeding on red clover at this station have a pH range of 5.8–6.8 and a temperature of 37°–39°C. The main end product of the action of the amylase on starch is maltose. Small amounts of glucose and maltotriose are also produced. Several α-amylases, e.g. salivary α-amylase (Roberts and Whelan 1951) and a rumen streptococcal amylase (Hobson and Macpherson 1952) produce mainly maltotriose with some maltose and glucose. Others, e.g. malt α-amylase and pig pancreatic α-amylase (Meyer and Gonon 1951) are reported to produce maltose and glucose. Epidinium α-amylase appears to belong to this latter group. The end products of the action of holotrich α-amylases on starch do not appear to have been described. Amylases normally do not attack intact starch grains (Turvey and Hughes 1958). Epidinium α-amylase does not attack intact potato-starch granules but appears to attack intact clover-starch granules. Extensive washing of the granules during preparation should have removed any disrupted granules. No explanation for the attack on clover-starch grains is at present possible although the smallness of the grains (1–2μ) may be a contributing factor.

The occurrence of a maltase in the extracts is not unexpected in view of the fact that maltose is the main end product of the α-amylase activity. By extracting the epidinia with water and phosphate buffer it is possible to extract these two enzymes separately.

The presence of two carbohydrases, both concerned with starch degradation, is useful confirmatory evidence for the observations of Oxford (1958) that starch grains are necessary for maintaining *in vitro* cultures of epidinia. The inability of the organism to use other carbohydrates is emphasised by the absence of the two major holotrich carbohydrases, β-glucosidase and invertase.

The above results together with those of Oxford (1958) seem to suggest that the activity of epidinia in the cow's rumen might depend on the amount of starch ingested by the animal. Of the two main components of dairy pastures in this country (clovers and grasses) clovers contain much more starch than grasses and their starch content can change from time to time (Bailey in press, 1958). At least one rumen disorder, bloat, is largely due to the consumption of clover by the animal. An investigation into the relationship of epidinia activity in the rumen and the changes in the amount of starch in clover in relation to bloat seems worthwhile.

ACKNOWLEDGEMENTS

My thanks are due to Dr A. E. Oxford of this laboratory for generously providing the epidinia cultures and to Miss J. Michael for technical assistance.

REFERENCES

BAILEY, R. W. 1958: Carbohydrates in Pasture Species. I. The Starch Content of Clovers and Rye-grass. *J. Sci. Fd. Agric.*

BOURNE, E. J.; MACEY, A.; PEAT, S. 1945: The Enzymic Synthesis and Degradation of Starch. II. The Amylolytic Function of the Q-Enzyme of the Potato. *J. chem. Soc.:* 882.

HOBSON, P. N.; MACPHERSON, M. 1952: Amylases of *Clostridium butyricum* and a *Streptococcus* Isolated from the Rumen of the Sheep. *Biochem. J. 52:* 671.

HOBSON, P. N.; PIRT, S. J.; WHELAN, W. J.; PEAT, S. 1951: The Enzymic Synthesis and Degradation of Starch. XIII. Improved Methods for the Fractionation of Potato Starch. *J. chem. Soc.:* 801.

HOBSON, P. N.; WHELAN, W. J.; PEAT, S. 1950: The Enzymic Synthesis and Degradation of Starch. X. The Phosphorylase and Q-Enzyme of Broad Bean. The Q-Enzyme of Wrinkled Pea. *Ibid.:* 3566.

HOWARD, B. H. 1957: Biochemical Studies of Individual Genera of Rumen Holotrich Protozoa. *Biochem. J. 67:* 18p.

JERMYN, M. A.; ISHERWOOD, F. A. 1949: Improved Separation of the Sugars on the Paper Partition Chromatogram. *Biochem. J. 44:* 402.

MEYER, K. H.; GONON, W. F. 1951: Degradation of Amylose by α-Amylases. *Helv. chim. Acta. 34:* 294.

MOULD, D. L.; THOMAS, G. J. 1957: Amylases of Holotrich Protozoa from Sheep Rumen. *Biochem. J. 67:* 18p.

OXFORD, A. E. 1958: Some Observations on the Culture of the Cattle Rumen Ciliate *Epidinium ecaudatum* Crawley Occurring in Quantity in Cows Fed on Red Clover (*Trifolium pratense* L.). *N.Z. J. agric. Res. 1:* 809.

ROBERTS, P. J. P.; WHELAN, W. J. 1951: End Products of the Action of Salivary α-Amylase on Amylose. *Biochem. J. 49:* lvi.

TREVELYAN, W. E.; PROCTER, D. P.; HARRISON, J. S. 1950: Detection of Sugars on Paper Chromatograms. *Nature 166:* 444.

TURVEY, J. R.; HUGHES, R. C. 1958: The Enzymic Degradation of "Raw" Starch Granules. *Biochem. J. 69:* 4p.

AN EXAMINATION OF THE SURFACE-ACTIVE AGENT PR 51 AS AN ACCELERATOR OF DRAINAGE FROM PASTURE TOPSOILS

By M. W. Gradwell, Soil Bureau, Department of Scientific and Industrial Research, Wellington.

(*Received for publication, 26 June 1958*)

Summary

The surface-active agent (surfactant) PR51 has been tested for its ability to accelerate the drainage of water from pasture topsoils.

Groups of tube infiltrometers 4 in. in diameter were placed on various soil types. Most tubes showed no response to the surfactant but the rate of infiltration increased in some members of each group. The mean gain in infiltration rate was about 20%. The moisture contents recorded in columns of artificially packed soil after a period of drainage from the saturated condition were decreased by about 1% on a dry-weight basis by the surfactant. A similar drying was recorded in the topsoil of Manawatu silt loam under winter grazing.

These gains in drainage are rather small to be of practical use to farmers.

Introduction

The deterioration of soil structure under winter grazing, or "pugging", is a major problem in the high-producing pastures of New Zealand. It is accompanied by a decline in topsoil permeability such that surface water lying in pools on the pasture after rain may take several hours to filter into the soil. Recently the surfactant PR51, a water-soluble alkylated aromatic sulphonate, produced by the Atlantic Refinery Company, has been reported as increasing the rate of infiltration of water into soil (Willis 1955). Increases of as much as 300% have been recorded (Slawson 1956). It seemed desirable, therefore, to test the application of these findings on infiltration to some New Zealand soils and also to carry out other tests designed to explore more fully the effect of the surfactant on soil moisture. The testing programme fell into three parts: (a) infiltration tests in the field and percolation tests on short undisturbed soil cores in the laboratory; (b) determinations of the moisture content of soil in artificially packed columns after a period of drainage from the saturated condition; (c) determinations of the moisture content and density of the topsoil of Manawatu silt loam under pasture in winter.

Methods

(a) Infiltration tests were carried out with thin-walled steel cylinders 6 inches long and of 4-inch diameter. These were driven vertically into the soil to a depth of 2 inches. Pasture plants in the area were cut short with scissors before the cylinders were driven through the soil surface and replicate cylinders were all placed within an area 10 ft in diameter. The cylinders were filled with water to a depth of approximately 3 inches above the ground

N.Z. J. agric. Res. 1: 834–840.

and the infiltration rate derived from the distance through which the water fell in a given interval of time. The interval for a particular cylinder was chosen to permit a fall of about 1 or 2 cm between two successive readings of depth. After the fall had been recorded, the water was brought back approximately to its original level before the measurement was repeated. The procedure for taking a depth reading was as follows: a wooden bar was laid across the top of the cylinder with one edge along a diameter marked on the sides of the cylinder. A mark on this edge of the bar indicated the centre of the cylinder. A steel rule, held vertically, was placed against this mark with its lower end touching the surface of the water and the depth of the surface in the cylinder read off on the ruler where it intersected the bar. The vertical alignment of the ruler was preserved by keeping its lower end parallel to its own reflection in the water. It was found that readings carried out in this manner were reproducible to ± 0.5 mm. If the rate of infiltration, as measured over three successive and equal intervals of time, was constant, the surfactant was added and readings taken over three more intervals of the same duration. Any gain in the mean rate of infiltration during the last three intervals over the former rate was attributed to the surfactant. The intervals chosen did not exceed 30 minutes for any test.

Percolation tests were carried out on blocks of undisturbed soil 3 inches long and $4\frac{1}{4}$ inch diameter taken with a hammered-in core sampler with removable liners. Samples were taken from the 2 to 5 inch depth under pasture, replicate cores being taken within an area 10 ft in diameter. Samples were taken within a chain of the sites where infiltration tests on the same location were carried out. The percolation tests were conducted with the base of the cores suspended in air rather than immersed in water, as in an ordinary permeability test. The perched water-table thus produced was thought to resemble more closely the drainage conditions in a "pugged" pasture, where a saturated surface soil containing only small pores overlies a less compact subsoil with many large pores containing air. Prior to the tests, each core sample was laid on a bench and a concentric cylinder 3 inches long and 3 inches in diameter was driven into its upper surface to a depth of $1\frac{1}{2}$ inches by a screw press. This cylinder was then filled with water to a depth of approximately $1\frac{1}{4}$ inches and the rate of percolation determined by procedures similar to those used in measuring infiltration rates in the field, except that the water surface was allowed to fall only 0.5 to 1.0 cm between readings. In cases where percolation was slow the tests were conducted in a constant temperature room; where the water fell at a rate less than 1 mm/hr the test was discarded as it was not considered that a reliable measure of percentage gain on such a small rate of flow could be obtained within a reasonable time. Five cores were thus rejected out of forty-five originally sampled.

(b) Drained moisture contents were determined for soil columns packed in perspex cylinders 10 inches long and 2.7 inches in internal diameter. The topsoils used for the work were air-dried, ground and passed through a 2-mm sieve. A double layer of cheesecloth was tied tightly over the bottom of the perspex cylinders and soil was poured into them through a funnel at a slow and steady rate, the cylinder being rotated meanwhile to prevent segregation of soil crumbs of different sizes. Pouring was continued until the soil column in the cylinder reached a height of 9 inches, the board on which the cylinder stood being vibrated meanwhile with a Syntron V-9 vibrator. The top of the column was smoothed to a horizontal surface and covered with a filter paper to protect it from channelling by water, 0.01 g of PR 51 being sprinkled on the filter papers in treated columns. 600 cm^3 of distilled water was then poured through each column and the top of the cylinder covered to reduce evaporation losses. As in the percolation test, the soil columns were suspended in air during their period of drainage. After 24 hours the 0–1, 1–2, 2–3, and 7–8 inch depths of the columns were carefully removed with a spoon and their moisture contents determined.

(c) The effect of PR 51 on the moisture content and density of Manawatu silt loam was tested in a winter grazing trial conducted by Grasslands Division, Department of Scientific and Industrial Research, on an area of new pasture 100 yards long and 22 yards wide, divided into 12 plots. Six plots were grazed by cows throughout the winter and the following spring, and six by sheep. At the beginning of winter the surfactant was sprinkled on three plots grazed by cows and three plots grazed by sheep at the rate of 30 lb per acre. The application was by hand to give as uniform a distribution as possible and was made when the pasture was short after grazing. On five subsequent occasions during the winter and spring, samples of soil for determinations of moisture content were taken from the $\frac{1}{2}$ to 2 inch depth at four sites in each of the twelve plots. On the last sampling date a repeat sampling was made on the 0 to 1 inch depth. Each sample for determination of moisture content comprised two cores taken 6 inches apart in a coring tube 1 inch in diameter. Apart from the restriction that no samples were taken from places where surface water was present the samples were randomly located. The density of the topsoil in late winter and spring was determined, for cow-grazed plots only, on two occasions. Undisturbed cores were taken from the 0 to 3 inch depth at four randomly located sites in each plot in the core sampler mentioned. Determinations of porosity were made on the 0.7 to 2.0 inch depth of these samples by driving into them, by means of a screw press, a thin-walled steel cutting cylinder 1.3 in. long and 3.3 in. in diameter. The soil at either end of the cylinder was carefully cut by hand to a plane surface as tested by a straight-edge. It was found on extracting the porosity cores from the cylinder that the intersection of its cutting edge with roots or soil crumbs harder than the surrounding soil had resulted in many small particles being torn from the cylindrical surfaces of the cores. As the cavities resulting were considered an unnatural formation likely to lead to high values of porosity they were plugged with remoulded soil taken from near the cores before the cores were oven-dried and weighed. The amount of remoulded material thus added to a core averaged 1% of its volume.

Results

TABLE 1. Percent Increases in Rates of Water Inflow into Soil Produced by the Surfactant PR 51

Location of Sample and Grid Reference	Soil Type	Increases in Laboratory Percolation Tests (per cent)	Increases in Field Infiltration Tests (per cent)
Taita, Hutt Valley N160: 512353	Heretaunga mottled silt loam	70, 0, 0, 0, 0	0, 0, 0, 0, 0, 10
Wharekohe Block, Whangarei County N19: 617933	Wharekohe silt loam	0, 60, 0, 120, 100	130, 180, 70, 30, 0, 0
Wharekohe Block, Whangarei County N19: 634938	Wharekohe silt loam	40, 0, 0, 0, 0	—
Gumtown, Whangarei County N20: 737025	Wharekohe silt loam	0, 0, 10, 75, 60	—
Bruntwood, Waikato, N65: 912422	Horotiu sandy loam	30, 0, 0, 0, 0	0, 0, 0, 0, 15, 0
Matangi, Waikato, N65: 912467	Horotiu sandy loam	30, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Patumahoe, South Auckland, N47: 372215	Patumahoe clay loam	10, 20, 0, 250, 0	0, 0, 0, 0, 0, 60
Karaka, South Auckland N47: 430272	Hamilton clay loam	15, 0, 50, 0, 90	180, 0, 30, 15, 0, 0

The results of the field infiltration tests and of the percolation tests on undisturbed cores are set out in Table 1. The rate at which the water surface fell in these tests ranged from 800 to 1.5 mm/hour, values lower than 10 mm/hour being found mainly for Wharekohe silt loam. The coefficient of variation of field infiltration rate was 137% for Heretaunga mottled silt loam, 128% for Wharekohe silt loam, 57% for Horotiu sandy loam and 64% for Patumahoe and Hamilton clay loams. In each group of replicate test results set out in the table the figures are arranged in order of decreasing inflow rate, i.e., the first figure is the percent gain in inflow of the core originally the most permeable, the second the gain of the second most permeable core and so on. It is seen that over the whole set of results there is no evident dependence of response to the surfactant upon the original value of permeability. Responses were limited to a portion of the tests only.

Moisture contents of artificially packed soil columns are set out in Table 2. It is seen that the surface soil of treated columns contained significantly less water on a weight basis after a period of drainage from the saturated condition. The porosities of the 0–3 in. depths averaged 62% and no consistent differences were found between the porosities of treated and untreated soil.

TABLE 2. Effect of PR 51 on Moisture Contents of Soil Columns after Drainage
(Moisture contents on a dry-weight basis: each figure the mean of six replicate columns)

	Depth (in.)	Moisture of Untreated Columns (%)	Moisture of Treated Columns (%)	Excess of Untreated Over Treated (%)
(a) Heretaunga mottled silt loam	0–1	53.0	52.5	0.5
	1–2	50.4	49.3	1.1*
	2–3	51.4	49.9	1.5*
	7–8	55.3	54.8	0.5
(b) Manawatu silt loam	0–1	51.3	50.0	1.3**
	1–2	47.3	46.7	0.6
	2–3	46.4	45.7	0.7
	7–8	50.2	49.8	0.4

* Difference significant at the 5% level of probability.
** At the 1% level.

Table 3 presents the results of the surveys of moisture content on a dry-weight basis carried out during the field trial. In the months following the application of the surfactant the treated plots gradually

TABLE 3. Soil-moisture Contents from Field Trial
(Moisture contents of ½–2 in. depth on a dry-weight basis percent; each figure the mean for 24 samples)

	20 June	22 July	25 August	22 Sept.	25 Sept.	25 Sept. (0–1 in. depth)
Untreated moisture	37.58	41.67	39.94	38.15	35.37	37.62
Treated moisture	37.91	41.05	38.97	37.00	34.10	36.30
Excess of untreated	−0.33	0.62	0.97(*)	1.15	1.27**	1.32**

(*) Difference significant at the 10% level of probability.

became drier than the untreated. In the final surveys the decrease in soil moisture produced by the treatment amounted to 1.3% and was significant at the 1% level. About the time when these significant differences in moisture content were recorded porosity determinations were made on the $\frac{1}{2}$–2 in. depth of the trial plots. No significant differences in porosity appeared between treated and untreated plots.

DISCUSSION

The effect of PR 51 on infiltration has been tested by overseas workers by the addition of the surfactant to half of the members of a group of closely spaced tube infiltrometers. Any significant differences between the treated and the untreated members of the group were noted. By this means it was shown that increases in mean infiltration occurred on some soils but not on others. Infiltration gains in individual tubes were not measured by this method. In the present work a method has been adopted which measures the gains in individual tubes. It is found that increases in percolation rate are produced by the surfactant in some members only of a group of tubes placed within a small area. It appears, therefore, that the response is governed by pore structure and that an increase in the rate of drainage of surface water produced by the surfactant would be attributable to faster inflow at some points only. The author considers that this faster inflow is caused by the removal of bubbles of air entrapped in the soil. A reduction of the surface tension holding bubbles below pore constrictions would allow some of them to float to the surface of the water. The mean gain in percolation rate over all tests was about 20%.

The reduction in drained moisture content produced by the surfactant in the field trial would be equivalent to a gain of 1.7% of air-space if the porosities of treated and untreated plots were equal. Air-space values recorded on this soil type in winter seldom rise above 5% (Gradwell 1956), so this gain is also, proportionately, a rather small one. Furthermore, a reduction of porosity in the treated plots to only 0.9% below the value in the untreated would nullify the air-space gain. Such a small difference in porosity would be difficult to detect. However, the values of treated and untreated porosity measured were nearly equal, hence it appears probable that some gain in air-space resulted from the treatment in the field trial. The probable gain in the laboratory trial with artificially packed columns was in the range 1.0% to 1.5% of air-space.

CONCLUSIONS

The surfactant PR 51 produces measurable acceleration of the drainage of some New Zealand soils, both by a faster rate of discharge of surface water and by more rapid reduction of the moisture content of the soil after surface water has disappeared. The improvements measured in the present work, however, appear rather small to be of practical use in draining wet pastures.

ACKNOWLEDGEMENTS

Acknowledgements are due to Mr. C. F. Sutherland, Soil Bureau, who assisted with soil sampling in Whangarei County; to Mr. D.

Edmond, Grasslands Division, Department of Scientific and Industrial Research, who organised the field trial on Manawatu silt loam and assisted with moisture surveys in the trial; to Mr. A. Glenday and Dr. J. H. Darwin, Applied Mathematics Division, Department of Scientific and Industrial Research, for statistical analyses; and to Mr. H. Woodyear-Smith, Challenge Phosphate Company, who assisted with sampling soils in the South Auckland and Waikato districts.

REFERENCES

GRADWELL, M. W. 1956: Effects of Animal Treading on a Wet Alluvial Soil under Pasture. *Proc. N.Z. Soc. Soil. Sci. 2:* 37-9.

SLAWSON, H. H. 1956: Oil Chemists Hear Surfactant Report. *Agric. Chem. Nov. 1956:* p. 93.

WILLIS, W. O. 1955: Freezing and Thawing and Wetting and Drying in Soils treated with Organic Chemicals. *Proc. Soil Sci. Soc. Amer. 19:* 263-7.

THE INFLUENCE OF DIET ON THE COPPER AND MOLYBDENUM CONTENTS OF HAIR, HOOF AND WOOL

By I. J. CUNNINGHAM and K. G. HOGAN, Animal Research Station, Department of Agriculture, Wallaceville.

(*Received for publication, 30 June 1958*)

Summary

The average copper content of hair of normal bovines was found to be 9 p.p.m.

Variations of dietary copper alone did not affect this, but increased molybdenum caused a reduction. Molybdenum content of bovine hair, bovine hoof, or sheep's wool was markedly increased by increased dietary molybdenum, but the increase was smaller or did not occur if dietary sulphate was also increased.

INTRODUCTION

The copper content of the hair of cattle has been suggested by van Koetsveld (1954, 1958) as a good indicator of the supply of copper in the diet. As hair is easy to sample, the possibility put forward by van Koetsveld of its use in diagnostic work has been further examined and this paper describes some studies of the effect of diet on the copper and molybdenum contents of cattle hair and hoof, and of sheep's wool.

EXPERIMENTAL

Hair of cattle was clipped from the flat area of the rump or over the ribs. Either the area was scrubbed with detergent and water and washed with water and finally with glass-distilled water before drying and clipping, or the area was brushed vigorously before clipping and the sample washed with detergent and finally with glass-distilled water before analysis. A suitable sample of hoof was obtained by cutting the tip from one toe, and, before analysis, all surfaces were pared so that no area exposed to soil was in the part analysed. Wool was washed in detergent and finally with glass-distilled water.

Copper was determined as described by Clare, Cunningham and Perrin (1945), molybdenum by Perrin's method (1946), and sulphate by the method of Johnson and Nishita (1952).

RESULTS

Normal Animals

This category comprised 23 Jersey heifers about 15 months old, living on pastures where no evidence of copper deficiency or molybdenum excess had ever been observed. The average value for samples from these 23 animals was 9.1 p.p.m. copper and less than 1 p.p.m. molybdenum in dry hair, and the range in values for copper was 7.7 to 11.2 p.p.m.

N.Z. J. agric. Res. 1: 841–846.

In 10 of these animals, copper in samples of rump hair averaged 8.9 p.p.m., and in hair from over the rib averaged 8.7 p.p.m.

Table 1 gives individual values for 10 of the animals and also shows colour of the hair and the liver copper determined from liver biopsy samples.

TABLE 1. COPPER IN LIVER AND HAIR AND COLOUR OF HAIR OF HEIFERS

Liver	Hair		
Cu p.p.m.	Cu p.p.m.	Mo p.p.m.	Colour
82	8.5	*	Black
109	9.1	*	White
44	9.0	*	White
42	9.0	*	Red
20	8.2	*	Red
29	7.7	*	Red
30	11.2	*	Brown
98	9.5	*	Roan
79	7.8	*	Fawn
42	9.4	*	Fawn
Average 58	8.9		

* = less than 1.

No relationship was displayed between colour of hair and copper content of hair in the animals in Table 1, nor was there any apparent relation between liver copper and hair copper. The absence of a relation between liver copper and hair copper was further evidenced in the results from another set of 10 animals of a similar age, in which the average hair copper was 9.5 p.p.m. and liver copper 234 p.p.m.

Experimental Diets

Analyses were made of hair from 5 groups of 4 Jersey cows held on a copper-deficient basal diet of oat grain and hay and supplemented with copper, molybdenum, or sulphate to give diets of the composition shown in Table 2. The supplements were supplied as drenches of solutions of copper sulphate, ammonium molybdate, or sodium sulphate, administered 5 days per week.

Samples of hoof were also examined and results are shown in the table.

The experimental feeding started in August 1954 with animals of yearling age, so the first hair samples were taken after the animals had been on the diet for 19 months, the blood samples after 21 months, and the second series of hair samples and the hoof and liver samples after 23 months.

TABLE 2. AVERAGE VALUES FOR COPPER AND MOLYBDENUM IN HAIR AND HOOF OF COWS ON DIETS OF KNOWN COMPOSITION

Group	No. animals	Diet			Hair				Hoof		Blood		Liver
					13.3.56		6.7.56		6.7.56		2.5.56		6.7.56
		Cu. p.p.m.	Mo. p.p.m.	SO_4 %	Cu. p.p.m.	Mo. p.p.m.	Cu. p.p.m.	Mo. p.p.m.	Cu. p.p.m.	Mo. p.p.m.	Cu. mg./100 ml.	Mo. mg./100 ml.	Cu. p.p.m.
1	4	4.0	*	0.1	8.1	1.7	9.6	1.8	4.4	*	0.071	0.027	25
2	4	4.0	15.5	0.1	5.8	9.5	5.3	11.9	5.0	3.2	0.055	0.178	9
3	4	9.0	15.5	0.1	9.4	10.0	9.6	15.6	5.5	3.3	0.093	0.135	50
4	4	4.0	15.5	0.55	4.6	1.6	4.9	1.5	4.5	*	0.065	0.019	9
5	4	9.0	15.5	0.55	7.6	2.1	8.6	2.1	4.3	*	0.093	0.019	81

* = less than 1.

The results in Table 2 do not show any effect of low copper alone on the content of copper in the hair. The low dietary copper in group 1 resulted in liver and blood copper contents that were below normal values, but the hair copper was close to the normal values given in the previous section of this paper.

On the other hand, there was a marked effect by extra dietary molybdenum or extra molybdenum plus sulphate in lowering the copper content of the hair. This lowering was prevented if copper was fed at the same time as the molybdenum or molybdenum and sulphate.

Increased dietary molybdenum caused a marked increase in the molybdenum content of the hair and this increase was prevented by additional sulphate in the diet.

The copper contents of hoof samples from the different groups were similar and thus showed no variation with the copper and molybdenum in the diets. The molybdenum contents of hoof, on the other hand, did vary and followed closely the pattern of response to molybdenum and sulphate in the diet that was displayed in the values for hair.

The blood and liver values serve to confirm the dietary experience of the experimental animals.

Sheep

Two groups of three weaner lambs about 8 months old were penned and fed a diet of chaffed oaten straw and crushed oats, in both of which, as the following figures show, the copper, molybdenum, and sulphate contents are low.

	Cu p.p.m.	Mo p.p.m.	SO_4 %
Oats	2.6	less than 1	0.03
Oaten chaff	3.0	less than 1	0.07

On 5 days per week supplements of copper and molybdenum were given to one group, and of copper, molybdenum, and sulphate to the other group, as drenches. Dosing commenced on 17 April 1956 and continued until 2 August. From 17 April to 12 June the supplements provided in each dose 30 mg Mo, 14 mg Cu, or 2 g SO_4. From 13 June to 2 August the copper and the sulphate were unchanged but molybdenum was reduced to 10 mg in each dose.

Before dosing commenced, the wool was closely shorn with fine electric clippers over a defined area on the flank. Samples were taken on the days shown in Table 3 and in each sheep the sample was taken from the same area on each sampling date. The wool analysed therefore represented that grown on the experimental diet since the previous sampling date.

This experiment tested the effect of increased molybdenum or molybdenum plus sulphate on the molybdenum content of wool. Results are given in Table 3.

TABLE 3. COPPER AND MOLYBDENUM IN WOOL AND BLOOD OF SHEEP

Supplements	Cu + Mo				Cu + Mo + SO_4			
Date Sampled	Wool Cu p.p.m.	Wool Mo p.p.m.	Blood Cu mg/100 ml.	Blood Mo mg/100 ml.	Wool Cu p.p.m.	Wool Mo p.p.m.	Blood Cu mg/100 ml.	Blood Mo mg/100 ml.
17.4.56		0.4	0.100			0.3	0.100	
30.4.56	13.3	4.7	0.120	0.286	13.0	2.9	0.109	0.067
29.5.56	9.7	13.9	0.108	0.370	11.7	5.5	0.103	0.098
13.6.56	9.3	13.7	0.103	0.261	9.1	4.7	0.105	0.047
6.7.56	8.3	9.9	0.100	0.213	8.6	2.6	0.089	0.023
20.7.56	9.2		0.113	0.198	11.3		0.093	0.021
2.8.56	9.6	7.2	0.159	0.180	10.6	4.3	0.148	0.027

The copper and molybdenum values for the blood showed the expected response from the supplements fed.

The increased molybdenum in the diet markedly increased the molybdenum in the wool, but this increase was very much smaller in the group to which sulphate was also given. The responses followed the same pattern displayed in molybdenum contents of the hair of cattle.

CONCLUSIONS

Our work has shown that the average copper content of hair of healthy cattle on pasture is 9.1 p.p.m., and this value is close to that found by van Koetsveld (1954, 1958). No association was found between the colour of the hair and its copper content, and in this we disagreed with van Koetsveld (1958) and Kikkawa *et al.* (1955) and agreed with the findings of Goss and Green (1955).

It was found that there was no close association between the copper status of the bovine as measured by the liver or blood copper, and the copper in the hair. On the other hand, an increase in dietary molybdenum caused a reduction in the copper of the hair and this reduction was prevented by increased copper in the diet.

The molybdenum in the hair was markedly increased by an increased dietary intake of molybdenum, whether or not extra copper was also ingested, but added sulphate in the diet prevented this increase in molybdenum of the hair.

The copper content of the hoof of cattle did not vary with varying copper or molybdenum in the diet. The molybdenum contents increased with extra dietary molybdenum, but this increase was prevented by additional sulphate.

The content of molybdenum in sheep's wool responded in the same way, as did that of bovine hair, to alterations in molybdenum and sulphate contents of the diet.

REFERENCES

CLARE, N. T.; CUNNINGHAM, I. J.; PERRIN, D. D. 1945: *N.Z. J. Sci. Tech. A26:* 340-50.

GOSS, H.; GREEN, M. M. 1955: *Science 122:* 330.

JOHNSON, C. M.; NISHITA, H. 1952: *Anal. Chem. 24:* 736.

KIKKAWA, H.; OGITA, Z.; FUJITO, S. 1955: *Science 121:* 43.

PERRIN, D. D. 1946: *N.Z. J. Sci. Tech. A27:* 396-405.

VAN KOETSVELD, E. E. 1954: *Tijdschr. Diergeneesk. 79:* 405.

—— 1958: *Ibid. 83:* 229.

INCIDENCE AND PARASITISM OF INSECT PESTS OF CRUCIFEROUS CROPS IN HAWKE'S BAY, WAIRARAPA, MANAWATU, RANGITIKEI, AND TARANAKI, 1956-57

By D. H. Todd, Entomology Division, Palmerston North.

(*Received for publication, 10 July 1958*)

Summary

Marginal damage to crucifer crops occurred in Hawke's Bay, Wairarapa, Manawatu, Rangitikei, and Taranaki during the 1956-57 season.

The virus disease was again a prominent factor in checking the white butterfly, *Pieris rapae* (L.), particularly during the latter part of the season when host populations were relatively high. No larval parasites, *Apanteles glomeratus* (L.), were recovered. Pupae collected were heavily parasitised by *Pteromalus puparum* (L.).

Sporadic attacks by the diamond-back moth, *Plutella maculipennis* Curtis, occurred in the Taranaki district. In other areas there was a low incidence of this pest. Larval parasites, *Angitia cerophaga* (Gravenhorst), were recovered from all districts. Parasitism by the pupal parasite, *Diadromus collaris* (Gravenhorst), was confined to the Manawatu, Rangitikei, and Taranaki districts.

No serious outbreak of the cabbage aphid, *Brevicoryne brassicae* (L.), was recorded.

INTRODUCTION

Analysis of the results obtained from a survey carried out in Hawke's Bay, Wairarapa, and Manawatu during the 1955-56 season, and reported by Todd (1957), showed a low incidence of insect pests of cruciferous crops in these districts, and in general, little crop damage occurred. Heavy parasitism of the white buttefly, *Pieris rapae* (L.), by *Pteromalus puparum* (L.), was recorded. However, a virus disease common to all districts was primarily responsible for the considerable reduction in white-butterfly population, particularly during the latter part of the season. The larval parasite, *Angitia cerophaga* (Gravenhorst), attained a high degree of control of the diamond-back moth, *Plutella maculipennis* Curtis. Only one serious outbreak of the cabbage aphid, *Brevicoryne brassicae* (L.), was recorded.

During the 1956-57 season a continuation and extension of the previous season's survey was undertaken, and field collections were made from 59 cruciferous crops growing in Hawke's Bay, Wairarapa, Manawatu, Rangitikei, and Taranaki districts. Observations made on the incidence of the pests in these districts during that season are detailed in the following account, and the percentage parasitism is recorded.

METHODS AND RESULTS

The methods and techniques used for collecting and handling larvae and pupae of white butterfly, and prepupae and pupae of diamond-back moth during the 1955-56 season, were again employed during the 1956-57 season. No attempt was made to assess the efficiency of *Diaeretus rapae* (McIntosh), as a controlling agent of the cabbage aphid, *Brevicoryne brassicae,* although this parasite was frequently seen in the field. The degree of aphid infestation occurring in each crop under examination was estimated visually.

In most cases, the procedure was again adopted of carrying out a preliminary survey primarily for the purpose of selecting suitable crops from which collections of insect pests could be made later in the season. In this instance, the period between the two surveys was reduced to a minimum. This was done to eliminate as far as possible the chances of a crop being fed off to stock before a collection could be made, and thus avoiding the extra work and time involved in finding alternative crops.

In mid February 1957, groups of crops were selected in Hawke's Bay and Wairarapa. Each group contained five crops. For making seasonal comparisons of the incidence and parasitism of crucifer pests in any one district, the groups were selected in close proximity to those chosen for the 1955-56 season. This also applied to the group of five crops in Manawatu, from which collections were made. During the first week in March 1957, four groups of crops were selected in the Taranaki district, each group consisting of five crops with the exception of group nine which contained four. No preliminary survey was carried out in either the Manawatu or Rangitikei districts prior to collections being made from crops growing in these areas.

Crop damage by white butterfly was classified as being:—

nil	— injury too slight to be of any consequence.
slight	— injury noticeable, but not appreciably affecting plants.
moderate	— injury conspicuous, plants partly defoliated.
severe	— injury conspicuous, plants completely defoliated.

Where white-butterfly damage is reported, it refers to the marginal part of the crop only. In most instances plant damage is highest towards the crop margins.

The areas surveyed during the 1956-57 season, and the distribution of the groups of crops from which collections were made, are shown in Fig. 1.

Between 19 and 21 February, thirty cruciferous crops growing in the Hawke's Bay and Wairarapa districts (groups 1 to 6 inclusive) were selected and examined. The majority of crops in these districts, apart from all crops in group 5 and a few crops in other groups, were in a wilted state owing to the generally dry conditions. Large populations of adult white butterflies were observed flying over crops, particularly in the Maraekakaho, Otane, Takapau Plains, and Masterton areas, and, in some cases, numbers had reached epidemic proportions. Eggs, and varying populations of larvae were found on most of the crops examined, but in no instance was the larval population great enough to affect seriously the whole of a crop. The plants most seriously affected were those confined to the margins, and in some

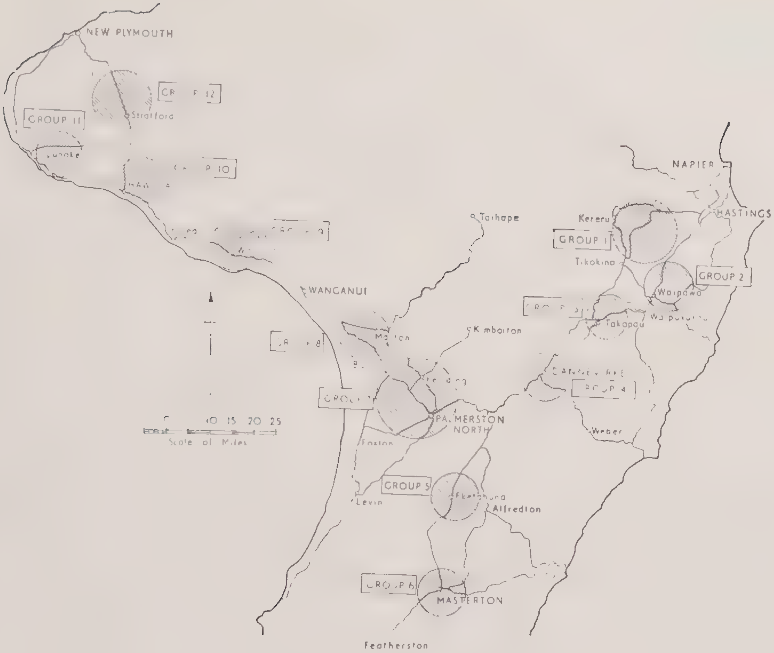

NEW PLYMOUTH
GROUP 11
Stratford
GROUP 1
GROUP 2
GROUP 4
GROUP 5
GROUP 6
NAPIER
HASTINGS
Taihape
Kereru
Tikokino
Waipawa
Takapau
WANGANUI
Kimbolton
Marton
Feilding
PALMERSTON NORTH
Foxton
Levin
Weber
Eketahuna
Alfredton
MASTERTON
Featherston
10 15 20 25
Scale of Miles

Fig. 1.—Areas surveyed and distribution of groups.

Group 1—Maraekakaho
,, 2—Otane
,, 3—Takapau Plains
,, 4—Dannevirke
,, 5—Eketahuna
,, 6—Masterton

Group 7—Palmerston North
,, 8—Marton
,, 9—Waitotara
,, 10—Hawera
,, 11—Opunake
,, 12—Stratford

cases many of these were completely defoliated. In no instance were severely defoliated plants found at a greater distance than 2 chains in from the edge. Generally, the crops showing the most damage and supporting the greatest number of larvae were those in groups 1, 2, and 3, with an injury classification of slight to severe. Injury to crops in groups 4 and 6 was classified as nil to moderate, and in group 5, as nil.

Virus-infected larvae were prevalent in all crops in groups 1, 2, and 3. Larval populations on crops in groups 4, 5, and 6, appeared to be relatively free from virus infection at this stage.

Diamond-back moth damage was not evident in any of the areas visited.

The cabbage aphid was present in most cases, but was insufficient in numbers to have any appreciable effect on the crops.

During the first week in March 1957, a preliminary survey was carried out in the Taranaki district, and 19 cruciferous crops growing in the Waitotara, Hawera, Opunake, and Stratford areas (groups 9–12 inclusive) were selected and examined. Most of the crops in the above areas, particularly those selected at Stratford (group 12) were in a reasonably healthy state and showing no tendency to wilt through dry conditions.

Populations of adult white butterfly varied considerably, with greatest numbers appearing over crops in the Waitotara and Hawera areas (groups 9 and 10) as well as over a number of crops in the Opunake area (group 11). Small numbers only were observed flying over crops visited in the Stratford area (group 12). One crop situated in the Opunake area (group 11) was showing moderate butterfly damage, but with this one exception, no large larval populations were recorded, and, in general, crop injury could only be classified as being nil to slight.

At this stage, virus-infected larvae were present on most of the crops in groups 9, 10, and 11, but no evidence of the disease was found on those in group 12.

With the exception of the crops in group 12, which appeared to be entirely free from any diamond-back moth damage, the remainder of those examined in the Taranaki district were damaged to some degree or other by this pest. Conspicuous damage to a number of crops in group 11 was recorded, particularly crop B in this group. A high percentage of the foliage was showing typical moth damage, and all stages of the pest were readily found.

Aphids, while present on all the crops examined, did not appear to be adversely affecting the growth of the plants.

Between 25 February and 1 March, field collections of white butterfly and diamond-back moth were made from the crops selected in Hawke's Bay and Wairarapa. During this survey it was observed that the incidence of virus-infected butterfly larvae had increased considerably since the preliminary survey carried out only a week earlier. This was particularly noticeable in crops which had previously been supporting the largest populations (groups 1, 2, and 3). A large proportion of the apparently healthy larvae, found on crops in these groups only a week before, were now practically one hundred percent infected, and in many cases in an advanced stage of putrefaction. Infected larvae were not found on all crops in groups 4, 5, and 6. However, it was obvious that the virus was becoming active in these areas and that it would spread rapidly. Although large numbers of adult butterflies were still in evidence over most of the crops, it was rather problematical at this stage whether any progeny would develop because of the high incidence of virus. Where possible, random collections of white butterfly pupae were made, and in most cases they were collected from either fence posts or grass stalks in the immediate vicinity of the crop.

There was still a low incidence of diamond-back moth in all the areas visited, and only small numbers of prepupae and pupae were collected.

No serious outbreak of the aphid *B. brassicae* was recorded, although the population levels of this pest on some of the crops visited was fairly high.

Field collections of white butterfly and diamond-back moth were made from crops in the Taranaki district (groups 9, 10, 11, and 12) between 12 and 15 March, approximately one week after the preliminary survey had been carried out in these areas. Of the very limited number of white-butterfly larvae found on the crops in the Stratford area (group 12) none appeared to be virus infected. Larval populations on crops in groups 9, 10, and 11 were also relatively small, but with a high proportion of larvae succumbing to virus infection. Owing to unfavourable weather conditions during part of this survey, normal activities of adult butterflies were interrupted, and in consequence it was not possible in every case to record their incidence. However, observations clearly indicated that adults at this stage were not particularly prevalent. Owing to the generally low incidence of white butterfly throughout the Taranaki district, it was only possible to collect a limited number of pupae.

Prepupae and pupae of diamond-back moth were collected from all crops with the exception of two in group 10, where none were found. Numbers found on individual crops varied considerably, but it was particularly noticeable that there was a much higher concentration of this pest in the Opunake area (group 11) where a number of crops were showing conspicuous damage. At the time of collection, it was observed that a high proportion of larvae and pupae on these crops were being attacked by a fungous disease, and it was apparent that considerable mortality of the pest was occurring. In all probability this fungus is *Entomophthora sphaerosperma* Fres reported by Robertson (1939), as being responsible for a high mortality of larvae and pupae at certain periods.

Aphid populations on the crops throughout the Taranaki district were generally small.

On 21 and 22 March, field collections were made from ten crops in the Manawatu and Rangitikei districts (groups 7 and 8). Severe butterfly damage had obviously occurred to a number of these crops at an earlier stage in the season. The damage was mostly marginal and badly defoliated plants appeared to be recovering and producing new leaf growth. Recovery of injured plants can be attributed to the virus which had almost completely wiped out the whole of the larval population. This was borne out by the fact that large numbers of decomposed virus-infected larvae were observed on the foliage of affected crops. Relatively large numbers of adult butterflies were still to be seen over most of the crops, the progeny of which, it was estimated, would become infected early in their larval development, and therefore die before causing any further crop damage. White-butterfly pupae were readily found on fence posts and grass stalks, and, in each case, random collections of 10-minutes duration were undertaken.

Diamond-back moth damage was practically nil throughout the Manawatu and Rangitikei districts, and only a limited amount of material could be collected.

None of the crops examined appeared to be adversely affected by aphids although in some instances isolated plants carried moderate to heavy populations.

TABLE 1. COLLECTION OF *P. rapae* LARVAE IN FIELD 1956-57

Group	Crop	Variety ‡	Date Collected	Duration of Collection (min)	No. Collected	Classification of Crop damage *	Group	Crop	Variety ‡	Date Collected	Duration of Collection (min)	No. Collected	Classification of Crop Damage*
1	A	C	26.2.57	10	0	3	7	A	C	21.3.57	10	0	3
	B	C	..	10	0	2		B	C	..	10	0	3
	C	C	..	10	0	2		C	C	..	10	0	3
	D	C	..	20	49	3–4		D	C	..	10	0	3
	E	C	..	20	46	4		E	C	..	10	0	3
2	A	C	25.2.57	20	11	1	8	A	C	22.3.57	10	0	3–4
	B	C	..	20	10	2		B	C	..	10	0	3–4
	C	C	..	20	3	3		C	C	..	10	0	3–4
	D	C	..	10	0	2		D	C	..	10	0	3
	E	C	..	20	59	3		E	C	..	10	0	3–4
3	A	C	26.2.57	20	62	2	9	A	C	15.3.57	10	0	1
	B	C	,,	20	65	3		B	S	,,	10	0	1
	C	C	,,	20	95	3		C	S	,,	--		2
	D	C	,,	20	39	4		D	C	,,	---		2
	E	C	,,	10	103	2							
4	A	C	27.2.57	20	83	2	10	A	S	14.3.57	10	0	1
	B	C	,,	20	123	1		B	S	13.3.57	10	0	2

	C	C, S	,,	20	67	2		C	C, S	14.3.57	10	0	2
	D	C	,,	20	52	1		D	C	15.3.57	10	1	2
	E	C	,,	20	42	1		E	C	14.3.57	10	0	2
5	A	C	28.2.57	20	15	1	11	A	C, S	12.3.57	20	5	2
	B	C	1.3.57	20	9	1		B	C	,,	—	—	3
	C	C	28.2.57	20	12	1		C	C, S	,,	20	11	2
	D	C	1.3.57	20	13	1		D	C	,,	20	50	2
	E	S	,,	20	10	1		E	C	,,	20	36	2
6	A	C	28.2.57	20	91	3	12	A	C	13.3.57	10	0	1
	B	C, S	,,	20	34	2		B	C	,,	10	0	1
	C	C	,,	20	56	2		C	S	,,	10	3	1
	D	C	,,	20	88	2		D	S	,,	10	1	1
	E	S	,,	20	35	2		E	C, S	,,	10	1	1

No parasitism by *A. glomeratus* was found in the survey.

Owing to the considerable amount of virus infection throughout most of the crops visited, the figures obtained were not for total population, but only for apparently healthy larvae.

* 1. Nil—injury too slight to be of any consequence.
2. Slight—injury noticeable, but not appreciably affecting plants.
3. Moderate—injury conspicuous, plants partially defoliated.
4. Severe—injury conspicuous, plants completely defoliated.

Assessment of crop damage is based on injury to plants growing in the marginal portion of the crop only.

‡ C = Chou moellier; S = Swede.

TABLE 2. FIELD COLLECTION RECORDS OF *P. maculipennis* PREPUPAE AND PUPAE INCLUDING PERCENTAGE PARASITISM* BY *A. cerophaga* AND *D. collaris* 1956-57

Group	Crop	Variety‡	Date Collected	Duration of Collection (min)	No. Collected	% Parasitism by *A. cerophaga*	% Parasitism by *D. collaris*
1	A	C	26.2.57	20	3	100.0	Nil
	B	C	,,	20	1	100.0	Nil
	C	C	,,	20	10	70.0	Nil
	D	C	,,	20	0	—	—
	E	C	,,	20	2	50.0	Nil
2	A	C	25.2.57	20	3	100.0	Nil
	B	C	,,	20	2	100.0	Nil
	C	C	,,	20	3	66.7	Nil
	D	C	,,	20	0	—	—
	E	C	,,	20	1	100.0	Nil
3	A	C	26.2.57	20	1	Nil	Nil
	B	C	,,	20	4	100.0	Nil
	C	C	,,	20	0	—	—
	D	C	,,	20	0	—	—
	E	C	,,	20	1	100.0	Nil
7	A	C	21.3.57	10	0	—	—
	B	C	..	10	2	50.0	Nil
	C	C	..	10	0	—	—
	D	C	..	10	0	—	—
	E	C	..	10	1	—	100.0
8	A	C	22.3.57	20	26	88.5	7.7
	B	C	..	20	47	90.2	9.8
	C	C	..	10	0	—	—
	D	C	..	10	1	100.0	Nil
	E	C	..	10	1	100.0	Nil
9	A	C	15.3.57	10	10	100.0	Nil
	B	S	..	10	4	75.0	Nil
	C	S	..	10	2	100.0	Nil
	D	C	..	10	9	66.7	11.1

4	A	C	27.2.57	10	6	100.0	Nil	10	A	S	14.3.57	10	3	100.0	Nil
	B	C	,,	10	0	—	—		B	S	13.3.57	10	0	—	—
	C	C, S	,,	10	0	—	—		C	C, S	14.3.57	10	18	33.3	16.7
	D	C	,,	10	4	100.0	Nil		D	C	15.3.57	10	1	—	100.0
	E	C	,,	10	6	100.0	Nil		E	C	14.3.57	10	0	—	-
5	A	C	28.2.57	10	3	100.0	Nil	11	A	C, S	12.3.57	20	65	92.3	Nil
	B	C	1.3.57	10	0	—	—		B	C	,,	20	95	92.6	4.2
	C	C	28.2.57	10	0	—	—		C	C, S	,,	20	22	50.0	18.2
	D	C	1.3.57	10	1	100.0	Nil		D	C	,,	20	71	45.9	Nil
	E	S	,,	10	0	—	—		E	C	,,	20	28	57.1	Nil
6	A	C	28.2.57	10	2	100.0	Nil	12	A	C	13.3.57	20	20	80.0	5.0
	B	C, S	,,	10	0	—	—		B	C	,,	20	22	50.0	4.5
	C	C	,,	10	0	—	—		C	S	,,	10	3	33.3	Nil
	D	C	,,	10	2	100.0	Nil		D	S	,,	10	2	50.0	Nil
	E	S	,,	10	0	—	—		E	C, S	,,	10	1	Nil	Nil

* Calculations based on the number of pupae found to be parasitised when microscopically examined.

‡ C = Chou moellier; S = Swede.

TABLE 3. PERCENTAGE PARASITISM OF *P. rapae* PUPAE BY *P. puparum* IN FIELD COLLECTIONS 1956-57

Group	Crop	Date Collected	Duration of Collection (min)	No. Collected	% Parasitism
1	A	26.2.57	10	48	100.0
1	D	,,	10	33	100.0
1	E	,,	5	125	93.6
2	A	25.2.57	10	53	90.6
2	B	,,	10	17	94.1
2	C	,,	10	108	100.0
2	D	,,	10	58	95.1
2	E	,,	10	40	97.5
3	A	26.2.57	5	98	96.9
3	B	,,	10	83	95.2
3	C	,,	10	47	87.2
3	D	,,	10	29	100.0
3	E	,,	10	51	74.5
4	A	27.2.57	10	33	100.0
4	B	,,	10	113	80.5
4	C	,,	10	42	95.2
4	D	,,	10	37	91.9
6	A	28.8.57	10	53	98.1
6	C	,,	10	29	89.6
6	D	,,	10	60	98.3
7	A	21.3.57	10	34	97.1
7	B	,,	10	114	99.1
7	C	,,	10	17	100.0
7	D	,,	10	14	92.9
7	E	,,	10	86	94.2
8	A	22.3.57	10	146	97.9
8	B	,,	10	86	97.7
8	C	,,	10	71	98.6
8	D	,,	10	64	93.8
8	E	,,	10	134	97.0
9	A	15.3.57	10	30	100.0
9	C	,,	10	31	100.0
9	D	,,	10	43	100.0
11	D	12.3.57	10	45	97.8

The results of field collections of white butterfly and diamond-back moth in Hawke's Bay, Wairarapa, Manawatu, Rangitikei, and Taranaki during the 1956-57 season are given in Tables 1, 2, and 3.

During early April 1957, a third and final examination of the crops growing in the Hawkes' Bay, Wairarapa, Manawatu, and Rangitikei areas (groups 1 to 8 inclusive) was carried out. The prime object of this survey was to confirm earlier predictions that the virus would ultimately eliminate larval populations of the white butterfly. It had also been thought that the majority of damaged plants in a crop would be capable of producing new leaf growth before being fed off to stock as winter fodder. Crops that had been showing severe marginal damage and supporting relatively large populations of virus-infected larvae only six weeks earlier were now entirely free from larvae, and injured plants were recovering rapidly.

A typical case of crop recovery is shown in Figs. 2 and 3. Fig. 2 shows a damaged chou moellier crop in the Tikokino area as it appeared on 26 February. Fig. 3 shows the same crop as it appeared on 8 April, only six weeks later. The most spectacular crop recoveries occurred where damage had previously been most severe.

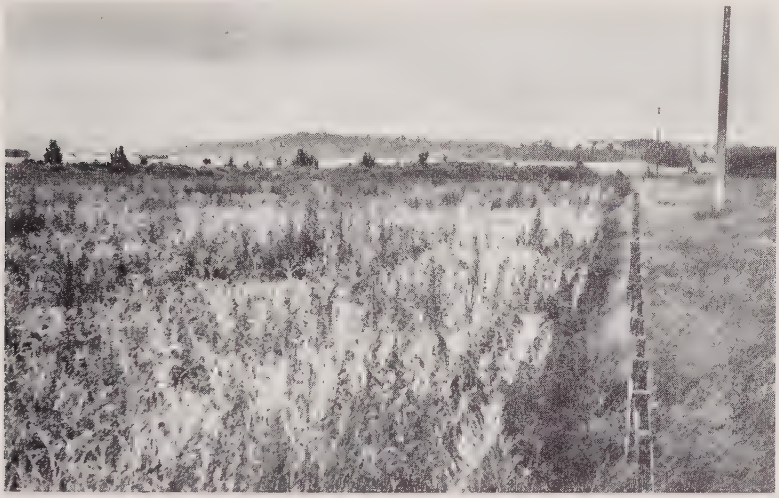


Fig. 2.—Severe butterfly injury to chou-moellier plants in marginal part of crop, photographed 26 February 1957.

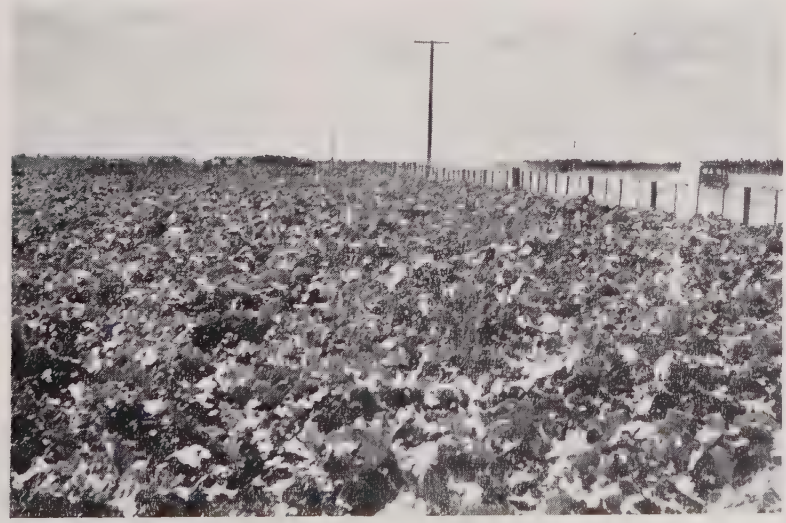


Fig. 3.—Same crop as in Fig. 2, photographed 8 April 1957, illustrating the rapid recovery of plants after virus had eliminated larval population.

DISCUSSION

A comparison of data obtained from field surveys carried out in the principal crucifer-growing areas in the North Island during 1955-56 and 1956-57, indicates clearly the general seasonal trend and behaviour of crucifer pests and their parasites in these areas.

During both seasons, observations have shown that white-butterfly populations do not reach significant proportions until about February. Any crop damage occurring at this stage is usually of a temporary nature, mainly because the virus is capable of eliminating larval populations of white butterfly with spectacular rapidity and effect. The pupal parasite, *P. puparum,* is particularly active towards the latter part of the season, and almost in every instance a high proportion of the pupae collected during this period are parasitised.

The field surveys carried out to date show that in general the diamond-back moth is not responsible for serious damage to crops growing in districts covered by the surveys. One exception to the generally low incidence of this pest was observed in the Opunake area (group 11) during the 1956-57 season. Conspicuous damage to crop B of this group was recorded, but, owing to the prevalence of fungus-infected larvae and pupae, on this and other crops in the same group, it can be assumed that the damage recorded would be temporary only. From the results presented in Table 2, which are comparable to those for the 1955-56 season (Todd 1957), it is clear that the larval parasite, *A. cerophaga,* attains a high level of parasitism in most districts.

The cabbage aphid, *B. brassicae,* did not present itself as a real problem to growers of cruciferous crops in any of the areas visited during the 1956-57 season. As was the case during the previous season, populations of this pest varied from crop to crop, but, probably owing to some climatic influence, it failed to reach significant proportions in any of the areas under survey.

For the 1957-58 season, it is proposed to carry out a further survey in all areas dealt with during the previous two seasons. This decision has been made with a view to covering as wide a range of conditions as possible. It is also hoped to pay particular attention to the development and incidence of the white-butterfly virus in relation to host populations and any other factors which may influence its development and spread.

REFERENCES

ROBERTSON, PHYLLIS L. 1939: Diamond-back Moth Investigation in New Zealand. *N.Z. J. Sci. Tech. A20:* 341-64.

TODD, D. H. 1957: Incidence and Parasitism of Insect Pests of Cruciferous Crops in Hawke's Bay, Wairarapa, and Manawatu, 1955-56. *N.Z. J. Sci. Tech. A38:* 720-27.

PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XIII. SUBSTANCES ISOLATED FROM CONCENTRATES OF FACIAL ECZEMA GRASS

By E. P. White, Ruakura Animal Research Station, Department of Agriculture, Hamilton.

(*Received for publication, 1 July 1958*)

Summary

A compound $C_{20}H_{30}O_6$, m.p. 149°c, containing two alcoholic —OH groups and a conjugated double-bond system, probably in a lactone ring, was isolated from toxic and non-toxic pasture in a yield of 1 in 50,000.

The substance responsible for the beaker test contains a major component of m.p. 260°c, probable formula $C_{26}H_{45}O_7N_3$. It was obtained from toxic pasture, the highest yield being 1 in 50,000. It gives, on hydrolysis, valine and leucine in a 1:1 ratio.

An impure material responsible for a false beaker test was isolated from pennyroyal.

The isoflavone formononetin was isolated from concentrates of a non-toxic pasture.

INTRODUCTION

This paper describes the techniques of isolation and some properties of four substances which have been found in ether extracts of dried mixed pastures, during fractionation work aimed at isolating the facial eczema poison (Part XII, White 1958).

Two of these compounds, not identifiable with any known compounds, occur in pasture at levels of 1 in 50,000 or less, on a dry-weight basis, and only small quantities have been obtained, sufficient to characterise them, and to get preliminary evidence on their structure. It is likely to be some time before quantities adequate for more structural work are available.

A third substance, not identifiable, and probably a mixture of related substances has been found in high concentration in pennyroyal, which was present in some pastures.

The fourth substance, isolated only from spring pasture containing red clover, has been identified as formononetin.

Substance m.p. 149°c

This is $C_{20}H_{30}O_6$, and is found in toxic and non-toxic ryegrass pastures. It follows the poison closely on adsorption columns. It is just below where at times some poison has been located on high-activity alumina, and is just above the poison on alumina V with benzene. This substance is appreciably soluble in water, and no more soluble in bicarbonate or sodium hydroxide, and gives no colour with ferric chloride, so it is not an acid or phenol. Two alcoholic —OH

N.Z. J. agric. Res. 1; 859–865.

groups are identified by the formation of a diacetyl derivative, m.p. 96°c, and reactions of a glycol or polyol are not given. The intense peak in absorption at 215 mμ (log ε 4.43 is indication of a conjugate double-bond system entirely exocyclic, or associated with a —C = O group (Gillam and Stern 1955). It gives no ketone reactions, and the absorption peak is too low in wavelength for a ketone. In the absence of a free —COOH group, the peak could be due to an αβ unsaturated ester or lactone, an example of the latter being dioscorine, with a peak at 217 mμ (log ε 4.21) (Pinder 1952).

The I.R. spectrum shows many peaks including 3413 cm^{-1} (—OH), 1624 cm^{-1} (conjugated double bond), and 1723 cm^{-1} (ester or acid carbonyl, or αβ unsaturated δ lactone). Pinder (1952) for δ-hexenolactone found 1725 cm^{-1}, many membered ring lactones giving 1741–1747 cm^{-1} (Anliker and Gubler 1957 , αβ unsaturated ketones 1635–1699 cm^{-1}. No typical lactone reactions were given, and results of saponifications did not give clear-cut products as might be expected from esters. It may be a dilactone complicated by additional —OH groups.

Substance m.p. 260°c

The impure substance has been isolated from four toxic grass samples in which it occurs at the most to 1 part in 50,000, and has been detected in other toxic samples from some of which it has been isolated in a less pure state in much lower yields. It has not been obtained from several non-toxic pastures. This substance is the basis of the "beaker test", and its occurrence in pasture samples has been correlated with toxicity by Perrin and Sandos (Part XIV, in press). In spite of a good correlation, there is no evidence for chemical alliance of it to the poison. The major component of the crude preparation is a substance of m.p. 260°c, probable formula $C_{26}H_{45}O_7N_3$.

During chromatography on high-activity alumina eluted with ether or ether/acetone, this substance occurs in or adjoining the zones where some poison has at times been detected, and traces of it follow the poison on other columns. The concentrates at 20,000 and 70,000 times may contain traces of it. It appears not to be a break-down product of the poison, and cannot be obtained from toxic concentrates by techniques known to destroy the poison. It gives no liver damage when fed at levels corresponding to over double the normal feeding levels, and is best isolated by processes known to destroy the poison.

Substance m.p. 260°c differs from the substance m.p. 149°c, and from the poison by a low solubility in organic solvents, and is insoluble in water and petroleum ether. There are no easily recognised substituents. Drastic acid hydrolysis gives two amino acids, identified on paper chromatograms as valine and leucine in a 1:1 ratio, and there is probably another unidentified amino acid. The subtance contains no free amino or carboxyl groups. Saponification gives a complex acid, hydrolysable to the same amino acids. Unless the third spot on paper is due to a dipeptide of valine and leucine, in which case another nitrogen, possibly amide, is to be accounted for, it seems likely that the substance is an ester or lactone.

The I.R. spectrum of a preparation of m.p. 244°c shows many peaks including 3375 cm^{-1} (—OH, or probably an inert >NH as there is no ready acylation), CH stretching frequencies at 2977, 2940 and 2888 cm^{-1}, and many peaks in the double-bond region, 1753, 1742, 1679, 1672, 1647, 1642 and 1532 cm^{-1}, some of which could be due to ester and peptide-like C = O groups. Characteristic peptide peaks are 3300, 1670, and 1650 cm^{-1} (Sutherland 1952), c.f. also similar I.R. peaks of cyclo-(glycylglycyl-DL-prolyl) (Smith 1957), and L-leucyl-L-prolyl anhydride (Johnson *et al.* 1951). Substance m.p. 260°c shows some resemblance to antibiotics built up of amino and hydroxyacids, such as the enniatins (Plattner *et al.* 1948; Cook *et al.* 1949), valinomycin (Brockmann and Geeren 1957; Brockmann and Schmidt-Kastner 1955), and amidomycin (Vining and Taber 1957), rather than to the cyclic peptides phalloidine (Wieland and Witkop 1940; Sorm and Kiel 1951) and gramicidin (Baron 1950).

The antibiotic amidomycin (Vining and Taber 1957), $C_{40}H_{68}O_{12}N_4$, is similar in properties to substance m.p. 260°c, and is water-insoluble and difficult to hydrolyse. It showed I.R. peaks at 1740 cm^{-1} (ester carbonyl), 1650 and 1540 cm^{-1} (amide I and II bands), 3300 cm^{-1} (>NH), and isopropyl bands at 1395 and 1375 cm^{-1}. Substance m.p. 260°c also shows bands at 1391 and 1378 cm^{-1}. Amidomycin is a 24-membered ring compound consisting of alternate D(—) valine and D(—)-α-hydroxy-isovaleric acid residues, linked by amide and ester linkages.

Substance from Pennyroyal

Routine beaker tests revealed in certain pastures an amorphous material resembling the m.p. 260°c substance in melting behaviour, in many chemical and physical properties, and in lack of characteristic absorption in the ultraviolet. It is found in pastures containing pennyroyal (*Mentha pulegium* L.) and was isolated from tops of pennyroyal in 0.11% yield. Several other labiates contain similar material. No compound of authentic purity has been isolated from this material. The crude substance shows I.R. peaks at 3410 cm^{-1} (—OH), 2920, 2855 cm^{-1} (C — C), 1697 cm^{-1} (C = O) and a few others.

Formononetin

The beaker test showed the presence of formononetin, an isoflavone of red and subterranean clover, in a non-toxic spring pasture. It can appear in the 3000 times concentrates of the poison. It occurred in the pasture to one part in 20,000 and was confined to the red-clover component, in which it occurred to one part in 500.

EXPERIMENTAL

Substance m.p. 149°c

Isolation. 6.10 kg of non-toxic grass was processed in 900 g lots to the 450 stage as described in Part XII. The residue in 70 ml of ether was put on an activity I-II alumina column, 12 by 2 in., and eluted with ether. The leading carotene zone was discarded, and the gap and the next yellow (xanthophyll) zone collected and evaporated. This was put on a 14 by 1 in. alumina column and the ether eluate of the colourless area before the xanthophyll zone collected, 200 mg of an orange gum containing crystals. This was dissolved in methanol and Darco carbon added until a colourless filtrate resulted. Evaporation, and washing with a little carbon tetrachloride, left 105 mg of crystals, m.p. 145–7°C. Recrystallisation from hot carbon tetrachloride containing some chloroform gave 77 mg of flat plates, m.p. 149°C. Other fractions of the columns gave none of this substance.

This substance has been obtained from another non-toxic pasture in a yield of 5 mg/kg, and from two toxic samples with yields of 5 mg and 13 mg/kg. It has also been obtained using activity I-II alumina with ether/acetone elution, when it is found in a faint yellow zone before the xanthophyll zone. More of it has been isolated from routine processing of toxic samples, by collecting the small yellow zone just above the cut for the poison on the alumina V column with benzene. Traces of it emerge from the magnesium carbonate column using ether just in front of the poison, and sometimes traces remain when a 70,000 concentrate is dissolved in a little carbon disulphide.

Properties. Readily soluble in most organic solvents, not in cold carbon tetrachloride or petroleum ether, slightly soluble in water to a neutral solution. It forms flat plates showing brilliant polarisation, m.p. 149°C $[\alpha]_D$ −103° (c, 0.781 in ethanol); u.v. absorption rises from 250 mμ to a peak at 215 mμ (log ε 4.43).

For analysis it was dried at 70°C, and 20 mm.

Found: C, 65.7, 66.0; H, 8.4, 8.2%; mol. wt. (Rast) 365, 385; no S, N (Dumas), halogen.

Found (another preparation): C, 66.0: H, 8.1%.

Calculated for $C_{20}H_{30}O_6$: C, 65.6; H, 8.2%; mol. wt. 366.

It gave no ketone reactions, no colour reactions with concentrated acids, no sterol or steroid reactions, no Legal test for acetone-like groupings, and no acrolein on $KHSO_4$ fusion (glyceride). It was recovered unaltered after refluxing with aqueous 1:5 hydrochloric acid, and was only partly altered on refluxing with methanolic KOH. It was not recovered from the alkaline solution after long refluxing with 50% aqueous KOH, was partly recovered when 25% KOH was used, and on acidification a substance which was not "149" was recovered. It is oxidised by lead tetraacetate and can be detected on paper chromatograms by this reagent, but the product does not give aldehyde reactions, and it does not reduce periodate or give reactions of polyols or carbohydrates.

Diacetyl Derivative. Refluxing with acetic anhydride with or without pyridine gave a glass, recrystallised from hot petroleum ether as large crystals of m.p. 96°C. It was dried at 30°C and 20 mm for analysis.

Found: C, 63.7: H, 7.5%.

Calculated for $C_{20}H_{28}O_6\ (CH_3CO)_2$: C, 64.0; H, 7.55%.

Substance m.p. 260°c

Isolation. 4.6 kg of a moderately toxic sample giving a good beaker test was processed to the 450 stage as for the poison, except that rotary evaporation was taken to dryness. The residue was dissolved in ether/acetone 70:30 and put on four 10 by 1 in. activity I-II alumina columns. Eluate up to the main orange zone was collected, giving on evaporation 847 mg of yellow gum. Treatment with a little ether, or in some cases methanol, left 41 mg of a white amorphous powder of m.p. 252°c, and later less pure crops totalling 22 mg. From the mother-liquors substance m.p. 149°c was isolated.

From 1.45 kg of another toxic grass the second yellow zone on alumina was collected using wet ether, decolourised in methanol with charcoal leaving 39 mg, and washed with a little carbon tetrachloride leaving 13 mg of crude material. Yields (crude material) from other toxic samples by similar methods have been 16 mg, 9 mg, 25.5 mg, and 12 mg per kg. A carbon column after alumina was used for the last preparation. It has also been obtained from residues of positive routine beaker tests.

Properties. Impure material of m.p. about 245°c was purified by recrystallisation from hot methanol (in which it is sparingly soluble), as very small needle clusters, m.p. 260–4°c. From 200°c a characteristic sublimate of very fine thread clusters forms in the m.p. tube. Sublimation at 200°c and atmospheric pressure gave a crystalline sublimate of m.p. 260°c, showing the same m.p. on admixture with the original substance. It is optically inert, and the u.v. spectrum shows only a rise below 250 mμ, with no peak to 207 mμ. For analysis it was dried at 90°c and 20 mm.
Found: C, 61.0; H, 9.0; N, 8.2, 8.2%; mol. wt. (Rast) 455, 475; no S.
Found for sublimed material: C, 61.0: H. 9.0%.
Calculated for $C_{26}H_{45}O_7N_3$: C, 60.9; H, 9.0; N, 8.1%; mol. wt. 511.

This substance is sparingly soluble in solvents, almost insoluble in ether, petroleum ether, carbon disulphide, and water. Ferric chloride in methanol gave no colour, it was recovered unaltered after refluxing with acetic anhydride, and gave no ketone reactions or colour with ninhydrin. The pure substance gave no colours with concentrated acids, and no colour tests for steroids. It was detected on paper by lead tetraacetate in benzene, but no aldehyde was formed.

On refluxing, or heating in a sealed tube for 15 h at 105°c with 1:5 hydrochloric acid, it was unaltered. With concentrated hydrochloric acid at 120°c in a sealed tube only a trace remained after 24 h. Nothing was extracted by ether or chloroform from the acid solution. The solution after evaporation and removal of acid by repeated evaporation with water was colourless and gave a ninhydrin reaction.

Paper chromatography (disc method) of the hydrolysate using butanol/acetic acid/water 4:1:5 (Partridge 1948), methylethylketone/ propionic acid/water 75:25:30 (Clayton and Strong 1954), and phenol/ water (Consden *et al.* 1944) by the ascending technique, gave two distinct zones on ninhydrin staining, identified as valine and leucine, and measurement of the colour compared with standards showed a 1:1 ratio. There was also a grey-stained zone immediately above the leucine spot, but this was not identified (R_f of the unknown 0.61, leucine 0.53, valine 0.42, methylethylketone system).

Paper chromatograms using systems for separation and detection of carbohydrates, organic acids, and bases revealed none in the hydrolysate. Similar hydrolysis to two amino acids as the only recognisable products resulted from refluxing with 30% sulphuric acid for 8 h.

It is insoluble in aqueous sodium hydroxide. Refluxing for 6 h with methanolic KOH gave no fraction extractable from alkaline solution, but on acidification an acid was extracted by chloroform or ether. This was a glass soon crystallising; on dissolving in aqueous sodium carbonate and acidifying, an oil formed, from which crystallised plate clusters of m.p. about 164°C, not obtained in adequate quantity or purity for analysis. It had a R_f of 0.97 in n-butanol/formic acid/water 47.5/2.5/13.5 (Lugg and Overell 1948). The aqueous phase on evaporation gave no more than traces of ninhydrin-reacting material. The acid on HCl hydrolysis gave the two amino acids and the grey-stained zone on paper.

Feeding. The following fractions from the preparation of substance m.p. 260°C were fed separately to guinea pigs without liver damage being detected—(a) 19 mg of the crude substance from 1100 g of toxic grass, (b) 80 mg of syrupy mother-liquors from (a), and (c) 80 mg of the total fraction from 890 g of grass.

Substance from Pennyroyal

Isolation. 500 g of tops of pennyroyal were concentrated to 450 times by the standard procedure, and a methanol solution put through a Darco/Celite column (2:1) 14 x 0.75 in., and the methanol eluate evaporated. Washing with 30% methanol left 0.55 g of amorphous solid. No means of separation into pure components has been found.

Properties. The crude product had a m.p. 220–230°C, with no sublimation. Analysis gave C, 72.0; H 8.9%; no N or ash. It showed u.v. absorption only below 230 mμ with no peak to 210 mμ, gave no characteristic colour reactions, and had no ketonic, acidic, or phenolic properties. It is insoluble in water, not eluted from high activity alumina by ether or ether/acetone, but easily eluted from low activity alumina by these solvents. Saponification gave only amorphous products.

Formononetin

4.29 kg of a spring pasture was put through the standard procedure to 450 times, and the fraction up to the xanthophyll zone obtained from activity I-II alumina using wet ether. The part sparingly soluble in methanol, 0.159 g, recrystallised from hot 90% ethanol (carbon) gave pure formononetin, colourless needles, m.p. 259°C. For analysis it was dried at 100°C and 20 mm.
Found: C, 71.4: H. 4.8%.
Calculated for $C_{16}H_{12}O_4$: C, 71.6; H, 4.5%.

The acetyl derivative had a m.p. 169°c (Reported for the substance, a m.p. 258°c; acetyl derivative 172–4°c; Bradbury and White 1951), and the u.v. absorption spectrum agreed quantitatively with that reported. It is readily distinguished from the substance m.p. 260°c by its phenolic properties.

ACKNOWLEDGEMENTS

Thanks are due to Dr. B. Cleverley, and Mr. I. R. McDonald, Dominion Laboratory, Wellington, for determination of I.R. spectra as paraffin mulls (149) or KBr discs (260), and for some advice on interpretation. Phenol/water chromatograms of amino acids were done by Mr. N. T. Clare.

REFERENCES

ANLIKER, R.; GUBLER, K. 1957: *Helv. chim. acta 40:* 119.

BARON, A. L. 1950: "Handbook of Antibiotics", p. 253, Reinhold, N.Y.

BRADBURY, R. B.; WHITE, D. E. 1951: *J. chem. Soc.:* 3447.

BROCKMANN, H.; GEEREN, H. 1957: *Liebigs Ann. 603:* 216-32.

BROCKMANN, H.; SCHMIDT-KASTNER, G. 1955: *Chem. Ber. 88:* 57-61.

CLAYTON, R. A.; STRONG, F. M. 1954: *Anal. Chem. 26:* 1362.

CONSDEN, R.; GORDON, A. H.; MARTIN, A. J. P. 1944: *Biochem J. 38:* 224.

COOK, A. H.; COX, S. F.; FARMER, T. H. 1949: *J. chem. Soc.:* 1022.

GILLAM, A. E.; STERN, E. S. 1955: "An Introduction to Electronic Absorption Spectroscopy". Arnold, London.

JOHNSON, J. L.; JACKSON, W. G.; EBLE, T. E. 1951: *J. Amer. chem. Soc. 73:* 2947.

LUGG, J. W. H.; OVERELL, B. T. 1948: *Aust. J. sci. Res. A1:* 98.

PARTRIDGE, S. M. 1948: *Biochem. J. 42:* 238.

PERRIN, D. D.; SANDOS, JEAN (in press): *N.Z. J. agric. Res.*

PINDER, A. R. 1952: *J. chem. Soc.:* 2236.

PLATTNER, PL. A.; NAGER, U. 1948: *Helv. chim. acta 31:* 594, 665, 2192, 2203.

SMITH, P. W. G. 1957: *J. chem. Soc.:* 3985.

SORM, F.; KEIL, B. 1951: *Coll. Trav. chim. Tchécosl. 16:* 366-79. (*Chem. Abstr. 47:* 3237).

SUTHERLAND, G. B. B. M. 1952: "Advances in Protein Chemistry", Vol. 7, p. 304, Academic Press, N.Y.

VINING, L. C.; TABER, W. A. 1957: *Canad. J. Chem. 35:* 1109.

WHITE, E. P. 1958: *N.Z. J. agric. Res. 1:* 433.

WIELAND, H.; WITKOP, B. 1940: *Liebigs Ann. 543:* 171-83.

THE EFFECT OF SEVERAL FUNGICIDES ON STEM ROT OF TOBACCO

By A. G. McLeod and R. Thomson, Tobacco Research Station,
Department of Scientific and Industrial Research, Motueka.

(*Received for publication, 26 May 1958*)

Summary

The importance of stem rot to the tobacco grower is discussed.

Two fungi, *Sclerotinia sclerotiorum* and *Botrytis cinerea* are the chief causes of the disorder.

Trials with fungicides in the seedling bed and in the field are described.

A good measure of control was obtained with ferbam, thiram, and dichlone.

INTRODUCTION

In the Motueka district 'stem rot' or 'collar rot' of tobacco has become a major cause of loss of seedlings at transplanting. This loss is of serious consequence to the grower. It can result in a shortage of seedlings and involves considerable time and labour in replanting at a period when other work requires to be done. As the replants will be later in maturity than the remainder of the crop, they result in a lack of uniformity in the tobacco which is reflected in uneven picking and curing, and usually an unduly high proportion of green leaf of low value, particularly if inexperienced labour is employed. For these reasons any measures that would control this disease in the seedling bed would be of economic importance to the grower. Several fungi are associated with the condition, but two, *Sclerotinia sclerotiorum* (Lib.) de Bary and *Botrytis cinerea* Fr., predominate and are the chief causal agents. There is a high degree of similarity in the symptoms produced by both fungi and as far as the grower is concerned the end effect is the same. Of the two, *S. sclerotiorum* is the more pathogenic. At the transplanting stage, infection is noticeable as a small blackish-brown lesion, sharply defined, at the base of the stem of the seedling. After planting in the field these lesions may increase in size until they encircle the stem and kill the plant. In the case of *B. cinerea* the lesion need not be as close to the base of the plant, and is more grey, with the margins not so clearly defined. In dying plants the causal agent is readily determined by the white cottony mycelium and sclerotial masses of *S. sclerotiorum*, or the greyish mycelium and conidiophores of *B. cinerea* (Fig. 1).

Losses are most severe during cool wet weather. With dry warm weather, plants may appear to "recover" but these "recovered" plants are easily blown over at maturity and constitute a source of infection for a later attack in the field should weather conditions prove favourable.

Laboratory isolations have consistently produced either *S. sclerotiorum* or *B. cinerea* alone, never both from the same plant. The

N.Z. J. agric. Res. 1: 866–873.

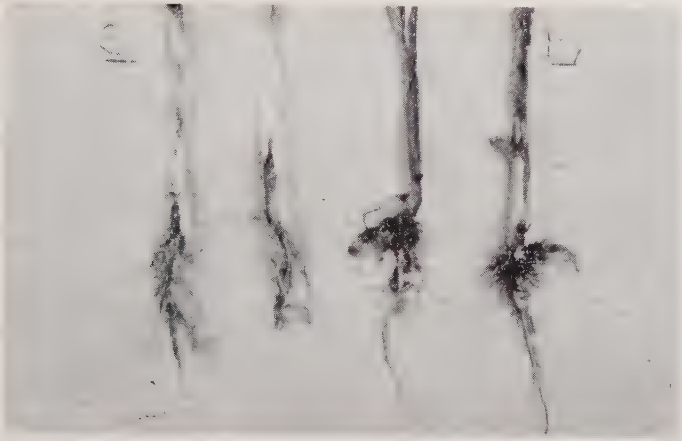


Fig. 1.—Stems of tobacco transplants attacked by *Sclerotinia* (left) and *Botrytis* (right).

sclerotial stage of *B. cinerea* has not been seen in the field but has been occasionally produced in the laboratory. It is a common practice to apply a drench of Bordeaux mixture or a Bordeaux substitute with a watering can at intervals of 10 to 14 days for the control of angular leaf spot (*Pseudomonas angulata*), and it would be an advantage if stem-rot could be controlled by the same drench or some modification of it.

Experimental

Seedling Bed Trials

Treatment of the seedling-bed soil prior to sowing, with steam, formalin, Semesan Bell, zinc oxide, copper sulphate, red copper oxide, chloropicrin, DD, or calcium cyanamide plus urea did not control the sclerotial stage of *S. sclerotiorum* (Cawthron Institute Annual Reports 1941-46).

At the Tobacco Research Station, trials were carried out in the 1956 and 1957 seasons. No worthwhile infection developed in 1956 so remarks will be confirmed to the 1957 trials.

Six seedling beds 24 ft x 4 ft were steam sterilised on 20 August by the inverted pan method, fertilised with a 3-8-8 (N-P-K) mixture at $\frac{3}{4}$ lb per sq. yd and sown with the Virginia Gold variety on 16 September.

Cultures of *S. sclerotiorum* and *B. cinerea* were grown on potato dextrose agar in petri dishes (20-25 ml/dish) in an incubator at 23°c.

A sclerotial-mycelial suspension of *S. sclerotiorum*, and a sclerotial-mycelial-conidial suspension of *B. cinerea* was prepared by maceration of the cultures (4 plates in 200 ml of water per bed) in a Waring Blendor for $1\frac{1}{2}$ minutes. This inoculum was made up to three gallons with water and applied to the seedling beds through a watering can. Three beds were inoculated with *S. sclerotiorum* and three beds with

B. cinerea.

Prior to inoculation with *B. cinerea* a quarter pound of green tobacco leaf, macerated with 200 ml of water in a Waring Blendor for 1½ minutes was applied with three gallons of water through a watering can to each bed to provide a suitable substrate for the development of the fungus.

The beds were inoculated three times, on 9, 14, and 25 October. At the time of the first inoculation the plants were in the four-leaf stage.

Each bed was divided into ten sections and eight fungicides were applied on 11 October and again on 30 October (Fig. 2). In each bed there were two control sections which received an equivalent quantity of water at each application.

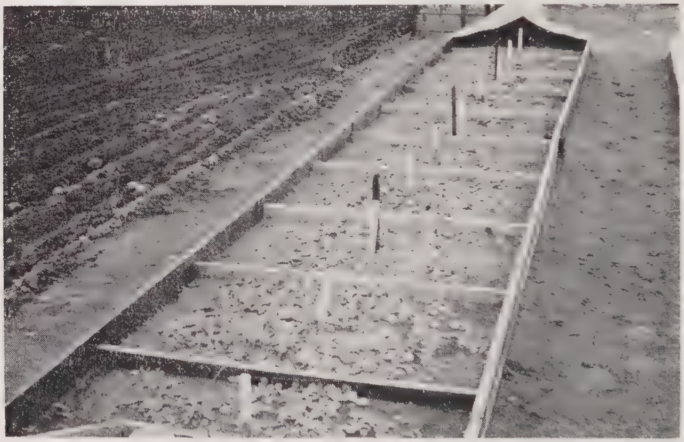


Fig. 2.—One of the beds in the seedling-bed trial.

The fungicides tested were:—

			oz/100 sq. ft	
Ferbam	70%	W.P.	3	
Zineb	65%	W.P.	3	
Polyram	70%	W.P.	3	(polyethylene thiuram disulfide)
Captan	50%	W.P.	1½	
Thiram	75%	W.P.	1½	
Peasan	70%	W.P.	1½	(quinonoxime benzoylhydrazone)
Copper oxychloride	50%	W.P.	7½	
PCNB*	20%	Dust	9	

*2nd application 3 oz/100 sq. ft.

With the exception of PCNB which was applied as a dust, the chemicals were mixed in water at the appropriate rate to give 3 gal per 100 sq. ft.

Isolations made from infected plants on 7 November confirmed the presence of *S. sclerotiorum* and *B. cinerea* in plots treated with zineb, Polyram, Peasan, and copper oxychloride and in the control plots. All plants were pulled and classified for degree of infection on 29 and 30 November for *Sclerotinia* and 3 and 4 December for *Botrytis.*

Results

Phytotoxicity. PCNB caused severe cupping (Fig. 3) and thiram a mild cupping of the leaves. These symptoms were visible three days after application. Fourteen days after the first application the distortion caused by thiram had disappeared. The distortion caused by PCNB did not disappear until 20 days after the first application. No distortion occurred following the second application of PCNB at a reduced rate. The second application of thiram induced a slight temporary chlorosis of the leaf.

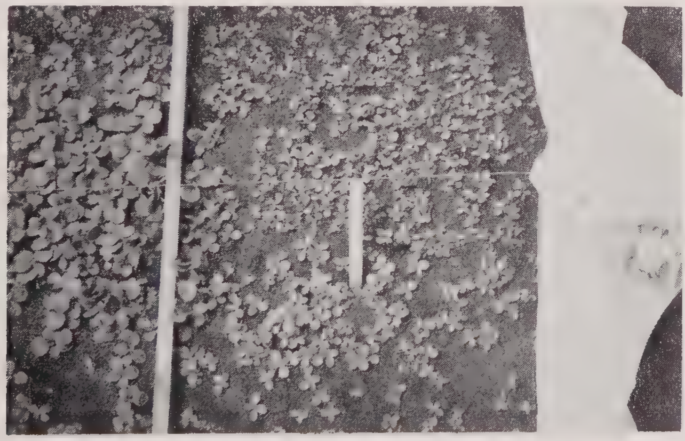


Fig. 3.—Cupping of leaves caused by PCNB; left, control; right, treated.

None of the other fungicides showed any phytotoxic effects at the strengths used.

Degree of Infection. All plants from each section were pulled and classified as:—

Clean—no infection.

Slight—basal lesion $< \frac{1}{2}$ in.

Heavy—basal lesion $> \frac{1}{2}$ in.

There was no difficulty in differentiating between slight and heavy infection as by the time the basal lesion had become more than $\frac{1}{2}$ in. long the stem was encircled, soft, and usually covered with mycelium of the causal fungus.

Statistical analysis of the data was made on the transformed mean of the percentage of clean plants in each case. The total number of plants examined, the transformed means, and the equivalent percentage of clean plants for the three replications of each fungus are shown in Table 1.

Controls A and B in each experiment show close agreement and were analysed as separates to illustrate the consistency of the results.

Ferbam and thiram treatments show a highly significant decrease in the amount of infection with either fungus over all other treatments. PCNB resulted in a worthwhile reduction in infection with both fungi, while captan gave a good control of *S. sclerotiorum* but not of *B. cinerea* infection.

Less than 1% heavy infection occurred with either fungus in the ferbam and thiram treatments. Heavy infection in the controls, copper oxychloride, zineb, Polyram, and Peasan treatments was approximately 10% with *Botrytis,* and 20% with *Sclerotinia.* Dead patches of plants amounting to approximately a quarter of the treated area occurred with these five treatments in beds infected with *S. sclerotiorum.* Very few plants were killed in the beds infected with *B. cinerea.*

TABLE 1. EFFECT OF SEVERAL FUNGICIDES ON STEM ROT INFECTION IN TOBACCO SEEDLINGS

	Sclerotinia			*Botrytis*		
Fungicide	Total No. Plants Examined	Transformed Mean	Equivalent % of Clean Plants	Total No. Plants Examined	Transformed Mean	Equivalent % of Clean Plants
Ferbam	1,259	83.0	98.5	1,383	84.4	99.4
Thiram	1,275	83.8	98.8	1,539	80.8	97.5
PCNB	1,337	62.8	79.1	1,417	30.8	26.2
Peasan	982	49.3	57.5	1,108	21.1	12.9
Captan	1,053	63.6	80.2	1,157	19.9	11.6
Copper oxychloride	928	42.6	45.8	1,040	14.5	6.2
Zineb	1,067	38.9	39.5	923	10.0	3.1
Polyram	759	39.5	40.4	893	9.0	2.5
Control A	975	46.0	51.8	1,060	15.5	7.1
Control B	1,118	46.7	52.9	1,200	15.9	7.5
Sig. diff 5% (between any two)		13.2			8.0	
1%		18.1			11.0	
Sig. diff 5% (from control)		10.8			6.6	
1%		16.0			9.8	

In 1956, trials were carried out with the co-operation of a grower who regularly experienced a severe attack of *Botrytis* in his seedling beds. A very shut-in humid location produced a favourable environment for the development of the fungus.

The following treatments were tried (lb/100 gal water):

Copper oxychloride	4
Thiram	1
Ferbam	2

These were all applied at 3 gal per 100 sq. ft.
Agromycin 200 p.p.m. at 10 gal per 100 sq. yd.
Cheshunt compound 1 oz to 1 gal per sq. yd.

Treatments were applied on 11 October, at which time the plants were about the size of a shilling. Seeding had been rather thick and small centres of infection were apparent throughout the bed. Plots were duplicated.

It was not possible to make counts from these beds but the results were quite spectacular. Ferbam definitely controlled the fungus, the plants bordering old centres of infection remaining quite healthy. No other treatment could be said to control the trouble although there appeared to be some slight reduction in the case of thiram. With ferbam there was a slight mottling of leaves but no retardation of growth. Thiram caused some cupping of the leaves and a temporary retardation of the plants. Agromycin resulted in a checking of growth with bleaching and burning of the leaves.

In the 1957 season another trial was laid down. However, a thinner seeding, along with better seedling-bed management in respect to ventilation and watering, kept the incidence of the disease so low that no results were secured.

Field Experiments

As stated previously, stem-rot can cause serious losses in the field at planting time, necessitating considerable replanting. Infection may originate from either of two sources. It may be carried over on plants from infected beds, or the causal organism may already be in the soil in the field. In 1956, preliminary trials were carried out on the property of a grower where stem rot occurred regularly, *Sclerotinia* being the predominating cause.

After the area had been planted, replanting was carried out with a transplanting "gun" which applied a quantity of water to the roots of the transplant. In a trial to determine whether stem rot could be controlled, certain fungicides were incorporated in the water used as follows:—

Ferbam 2 lb/100 gal.
Thiram 1 lb/100 gal.
Dichlone $\frac{1}{2}$ lb/100 gal.

A count of the replants 10 days after treatment gave the following figures for survival:

Dichlone	96%
Ferbam	84%
Thiram	82%
Untreated	50%

All fungicides reduced the amount of stem rot and resulted in a better stand.

The following season (1957) a trial to follow up these results was laid down in the field at the Tobacco Research Station. An area was prepared by planting out with seedlings, every one of which was moderately to severely infected with *Sclerotinia*. Most of these seedlings died back and developed a vigorous growth of fungus just below ground level. Those seedlings that did not die also showed good development of the fungus. On 11 December (nine days later) the whole area was replanted in the same plant locations with clean plants using the transplanting "gun". At this stage the area was divided into plots. The following fungicides were mixed into 100 gal water as before:

Ferbam 2 lb.
Thiram 1 lb.
Captan 1 lb.
Copper oxychloride 5 lb.

Dichlone was not included, as in another trial it had caused injury to plants.

One quarter pint of fungicide was applied with each plant; this would approximate 220 gal per acre. Controls received an equal quantity of clean water.

The trial failed in its original objective in that no stem rot developed in the transplants. Growth was most rapid in the controls, followed closely by ferbam, thiram, and captan. On the other hand copper oxychloride, at the rate used, killed almost 100% of the plants. It is difficult to account for the failure to secure infection on the transplants. Probably cultural operations produced an environment not favourable to the fungus, as the incidence of stem rot on the Station was very low while other growers were experiencing considerable losses. The mortality following the use of copper oxychloride is a feature that requires further investigation.

Discussion

Ferbam and thiram have given good control of stem rot in the seedling bed, and are the most efficient of the fungicides tested in the field. These results confirm the trend of preliminary glasshouse trials in which a number of fungicides were tested against *S. sclerotiorum, B. cinerea,* and *Pythium* spp.

Of the other fungicides tested, PCNB appeared promising and warrants trial of this material in a spray formulation. The remaining materials produced disappointing results, and three of them—copper oxychloride, zineb, and Polyram—showed a tendency (not significant) to increase stem rot. The use of copper oxychloride fungicides is general throughout the district and this material has given excellent control of angular leaf spot (*Pseudomonas angulata*) and has in the past been widely used as a protectant against stem rot. The high amount of infection with this treatment is difficult to explain, and it may be that the increase in stem rot over the past four seasons is due in part to the routine use of copper compounds which apparently increase the incidence of the disease.

Ferbam and thiram have both shown some phytotoxicity although any damage has been temporary and has not reduced the stand or the final size of transplants. As far as the writers know, neither ferbam nor thiram are effective against angular leaf spot.

Experience has shown the necessity of applying a fungicide which will control this disease and bed trials have demonstrated the effectiveness of Bordeaux mixture or a copper-based substitute. Recent work overseas had suggested that streptomycin might be used for this purpose but this material had shown considerable phytotoxicity under local conditions.

Future work with stem rot must also take into account that angular leaf spot is an important disease of seedling beds. The excellent protection given by copper oxychloride fungicides against this disease does not warrant the discarding of Bordeaux-type materials in the seedling-bed programme. Trials are planned to test these materials in combination with ferbam, thiram, PCNB, and dichlone. The possible phytotoxicity of the latter requires investigation.

Acknowledgements

We are grateful to Mr W. B. Taylor, Applied Mathematics Laboratory, for the analysis of the data; Mr R. W. Brien, PDD, who confirmed our identification of the causal organisms; Mr P. J. Wild who organised one field trial; and Messrs C. Harris and S. Silcock (growers) and Miss J. Newport who assisted with the work.

INFLUENCE OF APPLICATIONS OF SODIUM MOLYBDATE AND GROUND LIMESTONE ON A NEWLY SOWN PASTURE

By H. O. ASKEW, E. CHITTENDEN, JOYCE WATSON, and A. J. WATERS,
Cawthron Institute, Nelson.

(Received for publication, 11 July 1958)

Summary

On a newly sown pasture suitably treated with superphosphate four replications of applications of ground limestone at 0, 1, 5, 10, 20, and 40 cwt were set out; across these limed strips sodium molybdate at 0, 1, 2 and 4 oz per acre was applied, giving 24 treatments per replication.

At low rates of liming, sodium molybdate increased clover growth more than at high rates; there was no advantage in applying more than 2 oz sodium molybdate per acre. Increasing the rate of application of limestone improved the growth of the pasture even in absence of molybdate.

Three years after laying down, all plots carried the same amount of fodder in the spring. Implications of this result are discussed.

Nitrogen, phosphorus, and calcium contents of white-clover leaflets varied relatively little with treatment.

Manganese content was reduced by liming, but was little altered by variation in rate of application of molybdate. Manganese decreased as molybdenum content increased. A logarithmic relationship between manganese content and rate of use of limestone was found.

Molybdenum content was increased by successively larger applications of ground limestone in the absence of molybdate treatment, and by use of sodium molybdate. Logarithmic relationships between molybdenum content of white-clover leaflets and rate of application of sodium molybdate and also rate of use of limestone were found.

pH value and exchangeable calcium of the soil were increased by use of limestone, but citric-soluble phosphorus and potassium and exchangeable potassium and magnesium were reduced by such treatment. These results are important in relation to interpretation of analytical data in advisory work.

INTRODUCTION

In recent years the Moutere Gravels formation, a deposit of water-worn gravel, silt, and clay, has received a considerable amount of attention for reclamation to pastures from the cover of gorse, manuka, bracken, and other scrub plants with which a large amount of it is clothed. The process of reclamation has involved the initial use of ground limestone at rates up to two tons and of superphosphate up to five hundredweight per acre; in addition, good responses are obtained, in clover growth in particular, from applications of sodium molybdate. On this formation the virgin soils are acid and are lacking in phosphate, nitrogen, and exchangeable calcium; chemical analysis has shown too that the pasture growth is very low in molybdenum.

N.Z. J. agric. Res. 1: 874–895.

This particular geological formation therefore provides an opportunity to study the effect of applications of molybdate and of ground limestone on growth and chemical composition of any pasture established on it by the methods already mentioned. By reason of its naturally low status in calcium and molybdenum it provides also a means of examining over a period of years what may happen to a pasture when growth is stimulated by one or other of the agents used in reclamation: if use of molybdate encourages growth of clovers, which have a relatively high uptake of calcium, could the soil, in the absence of liming, be depleted of its calcium to a degree which would reduce growth, and also could use of limestone, in the absence of molybdate, artificially deplete the soil, already poor in this constituent, of its molybdenum? It is hoped that the present experiment may eventually give some answer to these questions.

In this paper are given chemical data obtained from samples of white-clover leaflets and of soils taken from an experiment where varying rates of sodium molybdate and ground limestone were used singly and in combination.

Experimental

The experimental area was situated on the top of rolling hill slopes with a northerly aspect on the east side of 88 Valley; the soil type is Rosedale silt loam, a strongly leached soil formed on the weathered Pleistocene Moutere Gravels formation in the Waimea County, Nelson. Previous to the time of preparation for a crop of ryecorn, grown in the autumn and winter of 1954, this land was in gorse and native grasses and had never been cultivated. This crop of ryecorn was fed off by sheep. After ploughing and cultivating with applications of further superphosphate to make a total of 5 cwt per acre, and treatment with ground limestone at 0, 1, 5, 10, 20, and 40 cwt per acre, the area was sown down with the farmer's grass and clover mixture at 45 lb per acre. At right angles to the strips treated with ground limestone, sodium molybdate was applied at 0, 1, 2, and 4 oz per acre. Four replications of all treatments were provided, with plots 5 yards square. The layout of the blocks within which a complete set of treatments was contained was such that Blocks A and B were on the upper side of the slope from Blocks C and D. Thus the latter two provided better growing conditions for the pasture, having probably slightly deeper soil, and being moister from drainage from the upper portion.

All treatments were on and seed sown by 28 September 1954.

In spite of a very dry summer, there was a good strike of grasses and clovers, although growth was very poor.

In each spring since establishment of the pasture, superphosphate, but no further limestone or molybdate, has been applied at 2 cwt per acre.

No samples were taken during the first year of establishment, but, from October 1955 onwards, periodical soil samples for determination of pH value, and spring and autumn samples of white-clover leaflets without petioles, were obtained for determination of their molybdenum, manganese, calcium (as CaO), phosphorus (as P_2O_5), and nitrogen contents. In one set of soil samples, exchangeable cations have been determined.

Molybdenum has been determined by the thiocyanate method after digestion of the sample material in a mixture of nitric, sulphuric, and perchloric acids. Manganese, extracted from the ash of the leaflets by digestion with 10% sulphuric acid, was estimated by the periodate method. Soils were ignited and the residue taken down to fumes with sulphuric acid, and the manganese then extracted by digestion with the dilute sulphuric acid. After ashing the leaflets at about 450°c and digesting with hydrochloric acid to render silica insoluble, the residue was taken up in dilute hydrochloric acid to form a solution from which aliquots were taken for calcium (by precipitation as oxalate) and for phosphorus (by precipitation as molybdate and titration in the cold of this precipitate with standard sodium hydroxide). Total nitrogen was determined by digestion of the sample with salicylic acid-sulphuric acid mixture, followed by distillation of the ammonia formed during the reaction.

RESULTS

Growth of Pasture

Unfortunately it has not proved possible to get as many yield data from these plots as was desirable. At about the time when the first samples of soil and pasture were taken for analysis, photographs were obtained of representative sods and of views of the plots. The sods, shown in Fig. 1, were from plots treated with the stated amounts (in cwt) of ground limestone but no molybdate. Fig. 2 shows the growth on a strip of plots receiving no molybdate but increasing quantities of ground limestone; it is a field view, taken on 31 October 1955, of the areas from which the sods of Fig. 1 were obtained.

In Fig. 1 the white clover plants receiving no ground limestone, and with 1 cwt, but to a less extent with 5 cwt, were stunted and poor in colour with pale-green leaves and reddish-coloured stems. Although clover plants were present they had not grown to any size. Liming at different rates had very marked effects on the growth of clover and grass. Even 1 cwt of limestone showed some benefit, while 5 cwt gave a substantial increase in growth. There was an even gradation of improvement in growth up to the 40 cwt rate. The photograph plainly shows the larger leaves of the clovers from the higher rates of liming. It is noticeable too that with increasing vigour of the clovers there is marked increase in development of grasses, especially ryegrass. The heaviest rate (40 cwt per acre) of ground limestone is not represented in Fig. 1: plants on this treatment were essentially the same as on the 20 cwt treatment. The field view in Fig. 2 indicates how bare areas seen in the plot in the foreground with no ground limestone disappear with increasing rate of application of the limestone; even with only 5 cwt per acre (the third plot from the front) clover growth has been fairly satisfactory. Whether the patchiness present is due to nodulation difficulties is not known; at any rate, under heavier liming, establishment of clovers was very satisfactory.

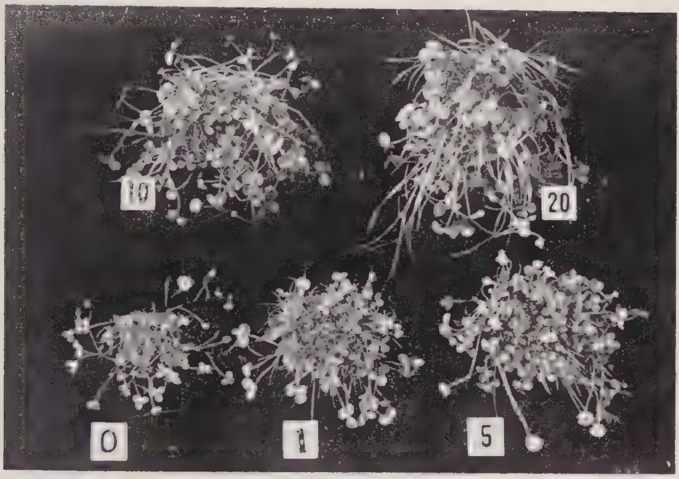


Fig. 1.—Representative sods from plots treated with varying amounts of ground limestone per acre. 29 Oct. 1955.

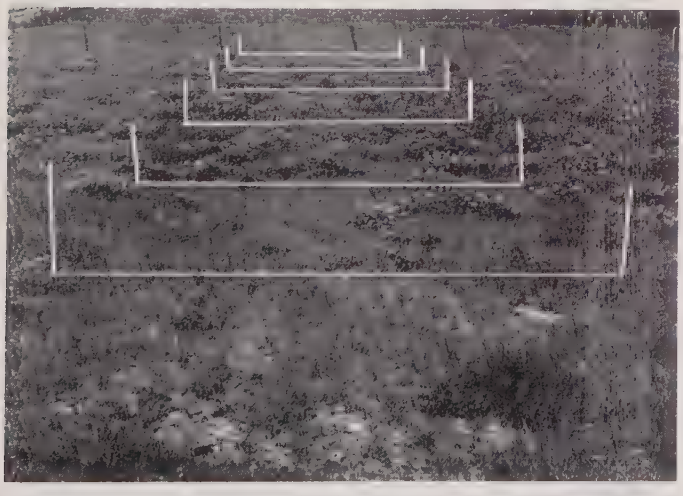


Fig. 2.—Area receiving no molybdate on 29 Oct. 1955. From front to rear the limestone treatments were 0, 1, 5, 10, 20, 40 cwt per acre respectively.

Another view is given in Fig. 3 where the plots on the left of the centre markers received no molybdate and those on the right were treated with sodium molybdate at 1 oz per acre. The plots in the foreground received 20 cwt per acre of ground limestone, those in succession beyond this receiving 10, 5, 1, and 0 cwt per acre. Under the heaviest rate of liming (40 cwt) there was no difference between plots receiving molybdate and those receiving none. At 20 cwt per acre, as shown by the plot in the foreground of Fig. 3, there was a slight difference in favour of the molybdenum-treated plot. But as the dressings of limestone decreased the effect of the molybdate become more marked, except where no limestone had been applied. In this last case, use of molybdate in the absence of limestone was not able to free the clover plants from the symptoms shown on the unlimed area. There was no advantage on any plot from increasing the molybdate from 2 oz to 4 oz per acre, but at the lower rates of liming 2 oz gave better results than 1 oz. It was possible therefore to see that under some circumstances a reduced amount of limestone in conjunction with molybdate could produce the same apparent bulk of fodder as was

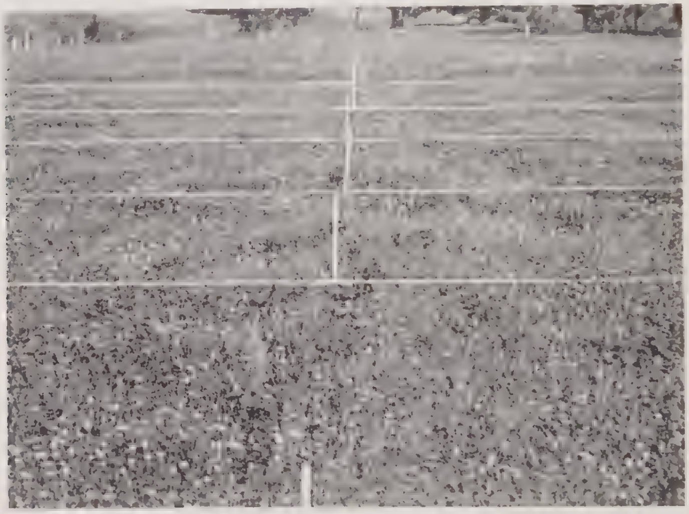


Fig. 3.—Left, no molybdate. Right, 1 oz sodium molybdate per acre. Foreground plot 20 cwt ground limestone per acre, passing to rear through 10, 5, 1, 0 cwt per acre respectively. Photo 29 Oct. 1955.

Fig. 4.—Unlimed area, as at time of cutting in November 1957.

produced by heavy applications of limestone in the absence of molybdate.

Yield of Fodder

Owing to various circumstances it has proved possible to obtain only one set of quantitative data for yield of fodder. These data, for fresh weight only, were obtained on 19 to 22 November 1957. By this time a most remarkable change (shown in Fig. 4) had taken place in pasture growth, the untreated plots and those limed at low rates being apparently as well-covered and as high yielding as those that had been more heavily limed. Thus the area did not have the appearance that might have been expected from Figs. 1 to 3, which showed the condition of the plots two years previously. In the intervening period the plots had been closed off to allow growth and then they were rapidly grazed by sheep. Over each winter no grazing, according to the farmer's usual practice, was carried out. This procedure had been adopted in the winter and spring of 1957, the plots being closed up at the beginning of spring. The material cut for weighing in November 1957 therefore represented growth from about the end of September to the time of cutting. The spring of this season was cold and late.

Blocks B and D (48 plots) were cut under good dry conditions on 19 November, but, owing to rain, Blocks A and C were not completed until 21 and 22 November; therefore the yield data represent two conditions from blocks cut when the fodder was dry or damp respectively. The data for Blocks A and C might therefore be expected, by reason of dampness and of having a slightly longer growth period, to give higher values than those of comparable plots of Blocks B and D. This expectation is found to be the case in the yield data of Table 1. Because of the number of plots concerned, and the very small differences in the weights of produce from individual plots (thus confirming the visual assessment of growth) the data have been grouped in two ways in Table 1: (1) taking molybdate as the variable and grouping yields from all limestone treatments for a given level of molybdate treatment, and (2) taking rate of application of limestone as the variable and grouping yields from the molybdate treatments.

In the upper section of Table 1 the differences between the weights of fresh material on the acre basis for the different treatments are quite small; the average yield is 16,781 lb per acre. When the molybdate plots are grouped together the data of the lower half of Table 1 are obtained. Again there is no evidence that the varying treatments have exerted any consistent effect on yield; indeed there is a tendency for the yield to fall with increasing amounts of limestone. This is particularly so for Blocks A and C, but not for Blocks B and D, where the variations in yield are very small and unrelated to treatment.

A further set of figures is given in the right-hand column of Table 1; this is the ratio of yields of Blocks A and C to Blocks B and D. If there were no effects from differences in moisture content of the pasture on the several treatments and there were no consistent soil differences between the blocks, this ratio should be relatively constant.

TABLE 1. WEIGHT (lb) OF FRESH FODDER FROM PLOTS RECEIVING VARIABLE RATES OF SODIUM MOLYBDATE AND GROUND LIMESTONE, NOVEMBER 1957

Material	Rate per acre	Block A	Block B	Block C	Block D	Totals	On acre basis	Wt. from A and C / Wt. from B and D
Sodium molybdate	oz							
	0	516.1	442.3	566.1	470.0	1994.5*	16,089	1.222
	1	525.9	448.1	601.9	474.9	2050.8*	16,543	1.186
	2	571.2	484.6	614.1	525.0	2194.9*	17,705	1.174
	4	547.9	418.8	567.2	547.3	2081.2*	16,788	1.151
Ground limestone	cwt							
	0	378.3	298.1	420.5	348.8	1415.7†	17,493	1.235
	1	402.6	285.6	401.1	348.0	1437.3†	17,391	1.268
	5	360.9	289.8	405.7	323.7	1380.1†	16,699	1.250
	10	357.4	313.9	404.2	321.7	1397.2†	16,906	1.198
	20	315.5	302.9	357.5	344.7	1320.6†	15,979	1.039
	40	346.4	303.5	360.3	330.3	1340.5†	16,220	1.115

* From 24 plots.
† From 16 plots.

For the upper section of the table the variations in the ratio could be well within the normal experimental error, but in the lower section the ratio tends to fall with increasing use of limestone, indicating that at lower rates the yields on Blocks A and C were relatively larger than at the higher rates. There is thus a suggestion that heavy liming has tended to reduce the effect of any fertility gradient between the upper and lower portions of the plots.

As stated above, the average yield per acre, calculated from all plots, was 16,781 lb. If the data for Blocks B and D, cut when dry, are taken, the average becomes 15,371 lb. This is possibly a truer measure than the overall average. If it is assumed that the dry matter will be one sixth of the fresh weight, then the yield of dry matter is approximately 2560 lb. This has been grown over about three months of the season and represents a very satisfactory production.

This area will continue under experiment so that further data will become available in later seasons.

Soil Analysis

Just over a year after the pasture was laid down, the first soil samples were taken on 27 October 1955, to a depth of 2 inches only, for determination of any pH changes that might have been brought about by use of ground limestone. As shown in Table 2, the untreated soil had a pH value of 4.6; this value was progressively increased to 5.3 with increasing amounts of limestone up to the maximum rate of 40 cwt per acre. Three months later, on 31 January 1956, the differences in pH value had become somewhat more regular, with the highest figure now at 5.65. Six more samplings were made, up to 18 April 1958, taking in these cases sets of samples from the 0–2 in. and the 2–4 in. depths. There are indications of progressive changes in the last two seasons, with a tendency for the pH values to be higher in autumn (April and May) than in spring. It is interesting that the pH values for a given treatment have varied relatively little over the two and a half years of sampling, even though there does appear to be a tendency for the pH value to increase on all plots over the last 16 months. The difference between the untreated and the most heavily limed plot has remained throughout at about 0.6 unit. A somewhat similar picture is seen in the data for the 2–4 in. depth so far as seasonal variations are concerned. Difference in pH value due to treatment has been very small, and it is only at the highest rate of liming that any definite increase in pH value has been brought about: even in this case the change is small. Penetration of calcium would appear to have been very minute.

TABLE 2. pH Value of Soil Samples from Plots Treated with Ground Limestone at Varying Rates

Depth of Sampling (in.)	Ground Limestone cwt/acre	Date of Sampling							
		27.10.55	21.1.56	4.5.56	14.9.56	24.12.56	17.5.57	29.10.57	18.4.48
0–2	0	4.60	4.65	4.90	4.70	5.05	5.10	5.00	5.25
	1	4.75	4.85	4.85	4.85	4.90	5.20	4.90	5.20
	5	5.00	4.95	4.95	5.05	5.00	5.00	5.10	5.25
	20	5.30	5.25	5.15	5.10	5.20	5.30	5.00	5.35
	10	5.20	5.40	5.15	5.15	5.20	5.45	5.40	5.60
	40	5.30	5.65	5.50	5.40	5.60	5.50	5.60	5.80
2–4	0	—	—	4.80	4.80	4.80	5.05	5.10	5.20
	1	—	—	4.95	4.90	5.00	5.10	5.20	6.00 (?)
	5	—	—	4.95	5.00	4.90	5.10	5.10	5.25
	10	—	—	4.90	4.90	4.90	5.15	5.00	5.30
	20	—	—	4.90	5.05	5.00	5.20	5.10	5.35
	40	—	—	5.10	5.00	5.05	5.40	5.00	5.40

The set of samples taken on 4 May 1956 from plots receiving ground limestone, but not molybdate, was analysed for citric-soluble phosphate and potash, loss on ignition, total nitrogen, and exchangeable cations (Table 3). Citric-soluble phosphate in the top sample was low in the untreated soil, and was still further reduced by the use of ground limestone; in the 2–4 in. sample the soluble phosphate was extremely low. Soluble potash was low also and was reduced in the topsoil by the applications of limestone as was found for phosphate, but there was no reduction in the 2–4 in. samples. Nitrogen contents and loss on ignition percentages were low and relatively constant, with little difference between the top and lower samples. Cation-exchange cations extracted by N neutral ammonium acetate solution were trebled but by reason of the application of the limestone the total exchangeable cations extracted by N neutral ammonium acetate solution were trebled in the topsoil in passing from the unlimed to the most heavily limed soil, while in the lower depth there was no consistent change due to liming, the average figure being 2.1 m.e.%. Of the exchangeable cations, calcium formed the major part; thus in the heavily limed soil calcium provided 92% of the total exchangeable cations, but only 69% in the untreated soil. In the lower depth, approximately two-thirds of the exchangeable cations was calcium. Magnesium level was satisfactory at about 0.8 m.e.% in both levels, and potassium was somewhat low, being only about 0.25 m.e.% in the upper level and 0.15 m.e.% in the lower. Both exchangeable magnesium and potassium were slightly reduced by liming. This is shown in Fig. 5 where the data for exchangeable calcium, magnesium, and potassium for the 0–2 in. samples are plotted against rate of liming; the depressing effect of the limestone on the last two elements is shown to be definite but slight. On the other hand exchangeable calcium is shown to be markedly increased in the top section but barely altered in the lower section. Base saturation has increased progressively at rates of 10 cwt, or more, of limestone per acre in the topsoil, but there has been no change in the low figure of 20% in the second depth.

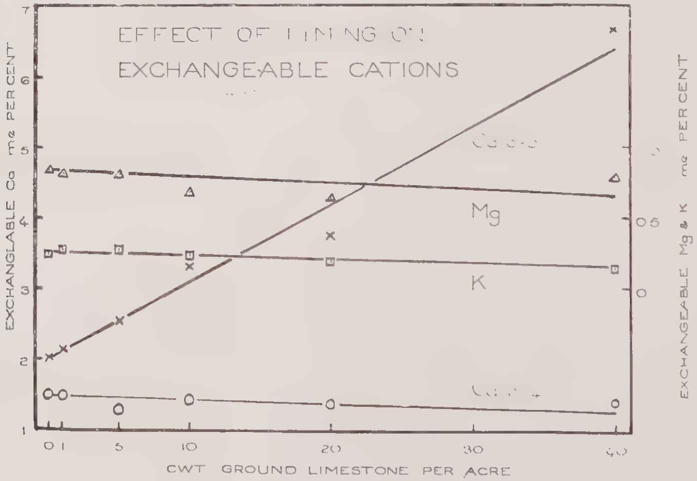


Fig. 5.—Effect of varying rates of use of ground limestone on exchangeable cations of soil.

Molybdenum, soluble in the tri-acid mixture, and the manganese extractable by taking down to fumes with sulphuric acid after ignition of the soil, were determined in the above samples. Results are given in Table 4.

The data of Table 4 show that the area was fairly uniform in respect of acid-soluble molybdenum at both depths of sampling; in all samples there was approximately 0.3 p.p.m. Although the figures for

TABLE 3. Chemical Data (dry basis) for Soil Samples from Pasture Plots Receiving Differential Liming
(Sampled 4.5.56)

Depth of Sampling (in.)	Limestone cwt/acre	Citric Soluble P_2O_5 %	Citric Soluble K_2O %	Nitrogen %	Loss on Ignition %	Exchangeable cations m.e.% C.E.C.	T.E.C.	CaO	MgO	K_2O	Saturation %
0–2	0	0.009	0.009	0.09	6.1	11.8	2.9	2.01	0.84	0.24	25
	1	0.004	0.003	0.09	5.2	11.4	2.85	2.09	0.80	0.27	25
	5	0.006	0.003	0.10	6.0	11.4	3.15	2.52	0.81	0.27	27
	10	0.002	0.006	0.11	6.5	11.2	3.9	3.30	0.68	0.23	35
	20	0.003	0.006	0.10	6.0	10.6	4.1	3.75	0.64	0.18	39
	40	0.004	0.007	0.12	6.9	11.2	7.4	6.85	0.76	0.14	66
2–4	0	0.001	0.005	0.10	6.3	11.6	2.4	1.49	0.86	0.15	21
	1	0.001	0.005	0.08	6.0	11.4	2.15	1.47	0.85	0.19	19
	5	0.001	0.006	0.10	5.9	11.7	2.2	1.26	0.74	0.17	19
	10	0.001	0.005	0.10	6.6	11.7	2.3	1.43	0.81	0.12	20
	20	0.001	0.005	0.08	5.5	10.2	2.0	1.29	0.68	0.14	20
	40	0.002	0.005	0.10	5.9	10.6	1.6	1.42	0.76	0.17	15

TABLE 4. Acid-soluble Molybdenum and Manganese p.p.m. of Dry Soil

Ground Limestone (cwt/acre)	0–2 in. Mo	0–2 in. Mn	2–4 in. Mo	2–4 in. Mn
0	0.27	96	0.25	69
1	0.28	69	0.23	67
5	0.27	79	0.27	79
10	0.27	91	0.30	71
20	0.30	93	0.27	84
40	0.32	73	0.30	79

the topsoil on the 20 and 40 cwt plots are slightly higher than those for the other plots it would not be safe to claim that the ground limestone had increased the acid-soluble molybdenum content. It will be shown later, though, that the available molybdenum as measured by uptake by the white-clover plants was increased appreciably at the higher rates of liming.

Manganese content has varied from 73 to 96 p.p.m. (average 83 p.p.m.) in the topsoil, and from 67 to 84 p.p.m. (average 75 p.p.m.) in the lower depth. The variations are quite unrelated to plot treatment. It will be shown later that the use of ground limestone has reduced the availability of the manganese to white-clover plants.

Analyses of White-clover Leaflets

Because of the great variations in the type and vigour of growth on the various plots it was considered to be more advantageous to take only white-clover leaflets for chemical analysis; this avoids difficulties in sampling due to variations in proportions of grasses to clovers, and in proportion of petiole to leaflet in the clovers. Four sets of samples have been taken, on: 21 October 1955, 4 May 1956, 4 April 1957, and 29 October 1957, thus covering two full years, but with the spring (October) sample of 1956 missing. It will be noted that the first samples were obtained about thirteen months after the pasture was sown.

Calcium. Data for the samples taken on 21 October 1955 and 4 May 1956 are plotted in Fig. 6, and those for 4 April and 29 October 1957 in Fig. 7. In the spring growth of 1955 there has been a distinct tendency for the calcium content to fall on lightly limed plots especially where molybdate has been used also; but with heavy liming combined with molybdate treatment, the calcium content was increased, although at no time did it reach the levels of those samples from plots where molybdate was not used. This effect is possibly due to the encouragement of extra growth by the molybdate for the nutrition of which only a limited amount of available calcium was present in the soil. By autumn (May) 1956 the effect of the liming was much more regular, although the actual calcium content was lower than in the corresponding spring samples. In the autumn of 1957 (Fig. 7) the effect of low rates of liming in raising the calcium content has become very marked, even the 1 cwt and 5 cwt applications leading to appreciable increases in calcium content, which was not however consistently increased at heavier rates: It is noticeable too that the 1 and 2 oz rates of molybdate have raised the calcium content more than has the 4 oz application. On 29 October 1957 the effect of the liming was still showing, but, except at the heaviest rate, calcium contents were no higher than those of the very low rates. At this period too the no-molybdenum plots tended to give higher figures than those which received molybdenum: any effect from rate of use of molybdate was inconsistent.

If the data are examined further it will be seen that for each set of four samples representing a stated limestone treatment, the variation in calcium content due to molybdate is small, being usually well within 10% of the average value for a set.

The important effects of small dressings of limestone in raising the calcium contents of the leaflets cannot be ascribed to differences in amount of clover on the several treatments, because, as has already been seen in discussing the yield data, the plots had become uniformly covered by the pasture. It would seem that the relatively small increase in exchangeable calcium, combined with the slightly increased pH value of the soil due to the small applications of limestone, have been sufficient to exert an important effect on the uptake of calcium by the clover plants.

Phosphorus. The average phosphorus content at each sampling was approximately 0.9% (as P_2O_5) and variations from this figure due to either limestone or molybdate treatment were rarely more than 10% of this value. As shown in Fig. 8 for the first two sets of samples in the

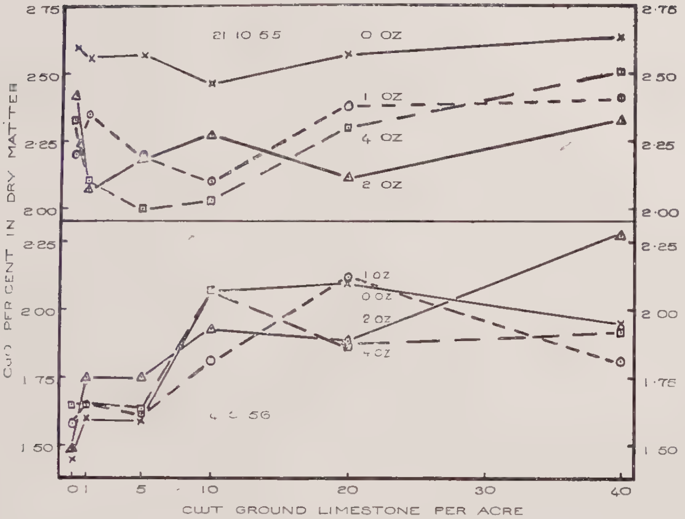


Fig. 6.—Calcium (as CaO) content of white-clover leaflets, on 21 Oct. 1955 and 4 May 1956, following use of varying amounts of limestone and sodium molybdate.

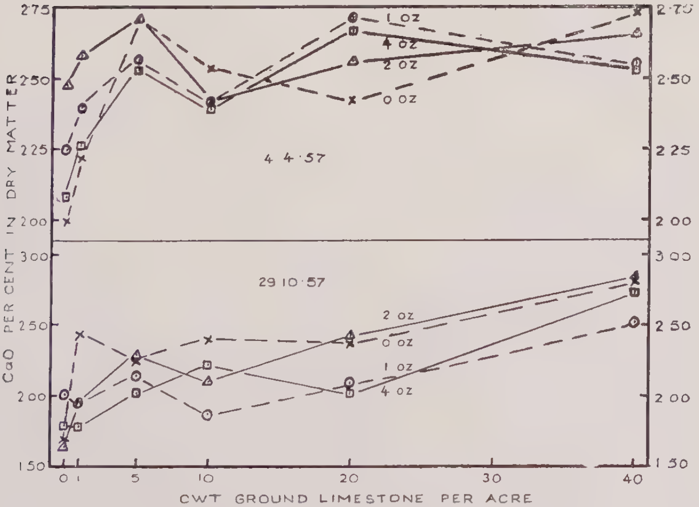


Fig. 7.—Calcium (as CaO) content of white-clover leaflets, on 4 Apr. and 29 Oct. 1957, following use of varying amounts of limestone and sodium molybdate.

spring, light liming, especially when combined with molybdate, increased the phosphorus content of the white-clover leaflets, but in the autumn of the same growing season light liming decreased the phosphorus content, which increased later, but then to decrease or remain constant, with heavy limestone treatment. At heavier rates of liming, use of molybdate increased the phosphorus intake, but not always in proportion to the amount of molybdate applied. In both parts of the season there was a distinct movement at the 40 cwt rate to approximately constant values, especially where molybdate was used; these values were lower than those found for the 20 cwt dressing. At both times the mean value was about 0.85% (as P_2O_5).

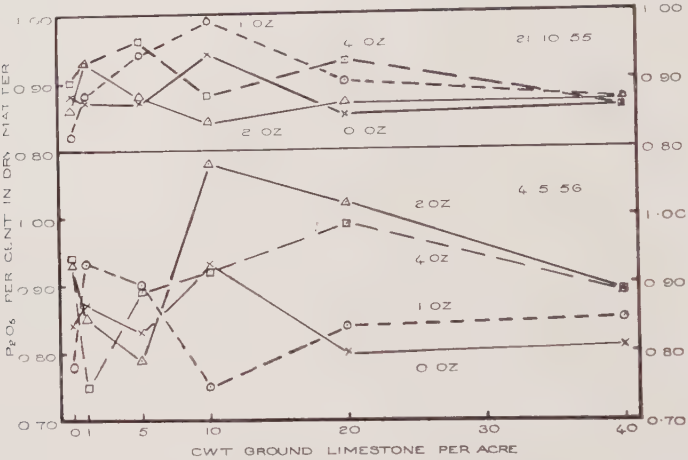


Fig. 8.—Phosphorus (as P_2O_5) content of white-clover leaflets, on 21 Oct. 1955 and 4 May 1956, following use of varying amounts of limestone and sodium molybdate.

In the 1956-57 season, samples were taken only in the autumn (4 April). Effects from both the limestone and molybdate treatments have been irregular but somewhat similar to those seen for the samples of 21 October in Fig. 8 (see Fig. 9). Again there was a tendency for a constant figure to be reached for the heaviest limestone dressing, but on this occasion the figure was only about 0.77% (as P_2O_5). The spring samples of 29 October 1957 have given results varying in a manner somewhat like those of May 1956 especially at the low rates of liming, where there was a reduction in phosphorus content. Except for the aberrant figure for the 1 cwt of ground limestone plus 1 oz of molybdate, the phosphorus content on 29 October 1957 varied relatively little with limestone treatment, and irregularly following use of molybdate (see Fig. 9).

The data of Figs. 8 and 9 suggest that two types of effect, especially at low rates of liming can occur: (1) increase in phosphorus uptake, as on 21 October 1955 and on 4 April 1957, or (2) decrease in phosphorus uptake, as on the other two sampling dates. What may govern the appearance of one result as against the other is not known. At higher rates of liming, the phosphorus intake is not necessarily greater than at low rates. In all cases use of molybdate may or may not increase phosphorus uptake.

When the data for corresponding sets of samples are examined, it is found that in the first two and the last sets, especially at low rates

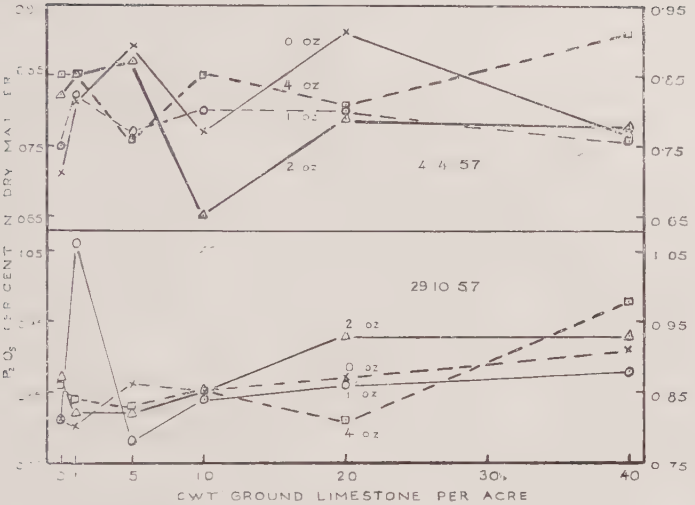


Fig. 9.—Phosphorus (as P_2O_5) content of white-clover leaflets, on 4 Apr. and 29 Oct. 1957, following use of varying amounts of limestone and sodium molybdate.

of liming, increase in phosphorus content was accompanied by a decrease in calcium, but that in April 1957 phosphorus and calcium rose together. At the high rates of liming this inverse effect is not consistently exhibited.

Nitrogen. Because both limestone and molybdate treatments, particularly in the first two years, have had an appreciable effect on the growth of white clover on these plots, it was of interest to see what changes may have occurred in the nitrogen contents of the leaflets. The analytical data for the four sets of samples are shown in Figs. 10 and 11. In all cases the nitrogen content was high, ranging from about 4.5% to 6.6%. In Fig. 10 the nitrogen figure was increased by liming up to a rate of 10 cwt per acre, and thereafter remained relatively constant, although individual figures have increased or decreased; in all cases however the proportionate changes have been small. Especially at the low rates of liming, and on unlimed plots, use of sodium molybdate has increased the nitrogen level in the leaflets; this is particularly marked in the set of samples of 29 October 1955, where each increase in application of molybdate has given an increase in nitrogen content.

In the data for the last two sets of samples, shown in Fig. 11, use of limestone in small quantities has tended to reduce the nitrogen content below those found for the unlimed plots; even with the heavy rates of liming the nitrogen contents have not risen consistently above an average figure obtainable from the data for the unlimed plots. It was again notable that molybdate was more effective in raising the nitrogen content at low than at high rates of liming.

When the data for nitrogen are compared with those for phosphorus and calcium it is found that nitrogen and phosphorus tend to vary in

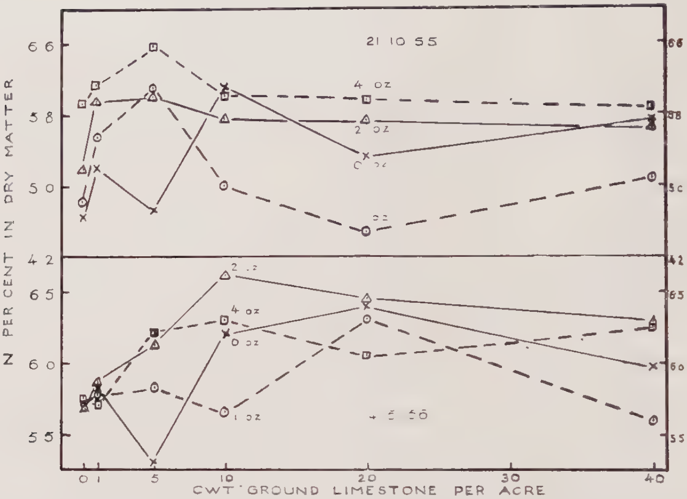


Fig. 10.—Nitrogen content of white-clover leaflets, on 21 Oct. 1955 and 4 May 1956, following use of varying amounts of limestone and sodium molybdate.

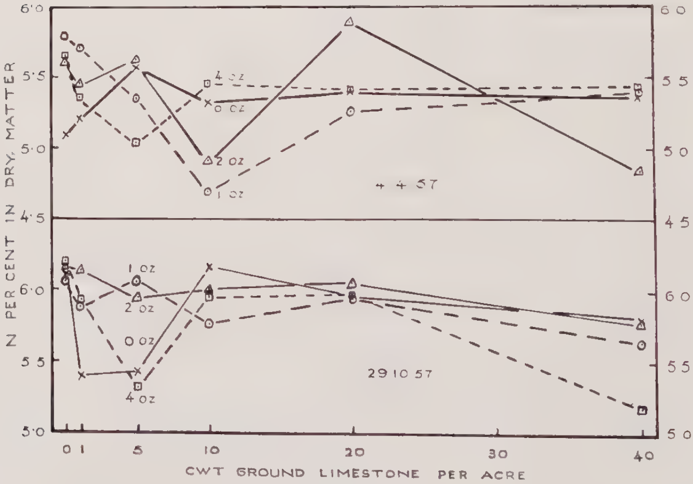


Fig. 11.—Nitrogen content of white-clover leaflets, on 4 Apr. and 29 Oct. 1957, following use of varying amounts of limestone and sodium molybdate.

the same direction, whereas nitrogen and calcium vary inversely. Further, in any set of samples where calcium is relatively low, nitrogen is relatively high throughout.

Molybdenum. The symptoms shown by the clover plants on the unlimed and lightly limed areas were indicative of a deficiency of molybdenum, combined possibly with a lack of nitrogen. However, the nitrogen content of the plants showing the most intense symptoms, from the unlimed area receiving no molybdate, on 21 October 1955, was 4.65%, a relatively high figure. For this material the molybdenum content was only 0.02 p.p.m., a very low figure. Following the use of ground limestone, the molybdenum content, in the absence of applications of sodium molybdate, was appreciably raised, as is shown by the lowest line in Fig. 12. Treatment with sodium molybdate very markedly raised the molybdenum content in proportion to the amount of molybdate used. Thus on the unlimed plots the molybdenum content of the clover leaflets was raised to 0.40 p.p.m. when 4 oz of sodium molybdate per acre was applied. As Fig. 12 demonstrates, the effectiveness of the 4 oz of molybdate in raising the molybdenum content of the fodder was markedly increased by liming, the figures at the 20 cwt and 40 cwt rates being 0.54 p.p.m. and 1.32 p.p.m. respectively.

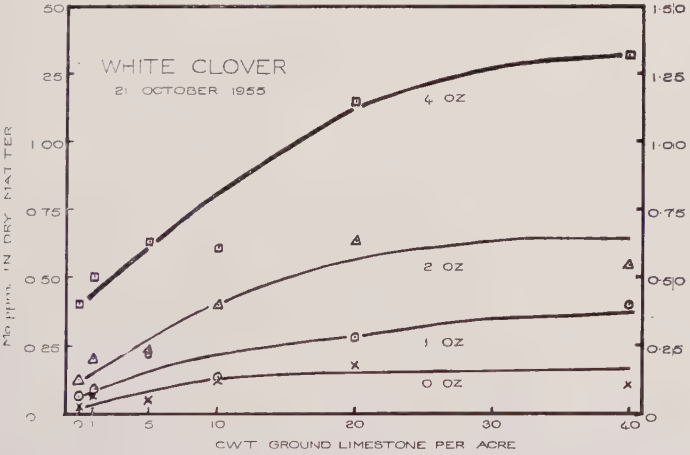


Fig. 12.—Molybdenum content of white-clover leaflets on 21 Oct. 1955 from varying amounts of limestone and sodium molybdate.

Data for the other spring sampling, on 29 October 1957, are shown in Fig. 13. On this occasion the molybdenum contents of the unlimed and lightly limed areas were somewhat higher than at the first sampling, being now slightly more than 0.1 p.p.m., still a low value. The heaviest rate of liming has nearly doubled the molybdenum content. The dressings of molybdate are shown to be still as effective three years after application, with the same increase in effectiveness due to liming, as was found on the first occason.

The data for the two autumn samplings may be considered together; they are shown graphically in Figs. 14 and 15. In these figures the same type of increase in molybdenum content with the heavier rates of liming occurred as was found for the spring samples, but two further features are present: firstly, these autumn samples are generally richer in molybdenum than the corresponding spring ones, even where no molybdate has been applied, and secondly there have been depressions in molybdenum content at the 1 cwt rate of application

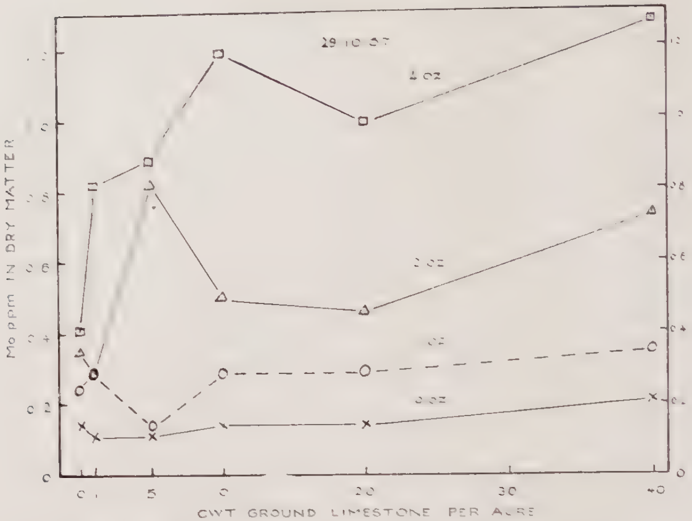


Fig. 13.—Molybdenum content of white-cover leaflets on 29 Oct. 1957.

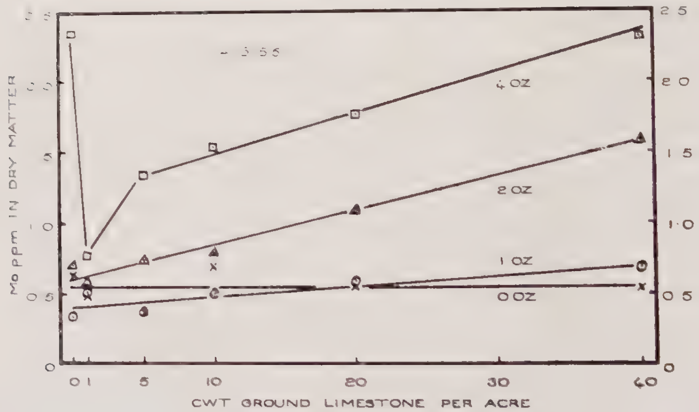


Fig. 14.—Molybdenum content of white-clover leaflets on 4 May 1956.

of ground limestone where molybdate has also been used. This depression is particularly marked in Fig. 15 but is present also in Fig. 14 for the higher rates of molybdate. Even in Fig. 13, for the spring samples in 1957, there is a suggestion of reductions in molybdenum content following use of small applications of limestone.

Manganese. On the acid soil of this experimental area it was to be expected that a high manganese content of the fodder, reducible by the application of ground limestone, would be found; moreover it was also of interest to see what the effect of the molybdate dressings would be on the manganese content.

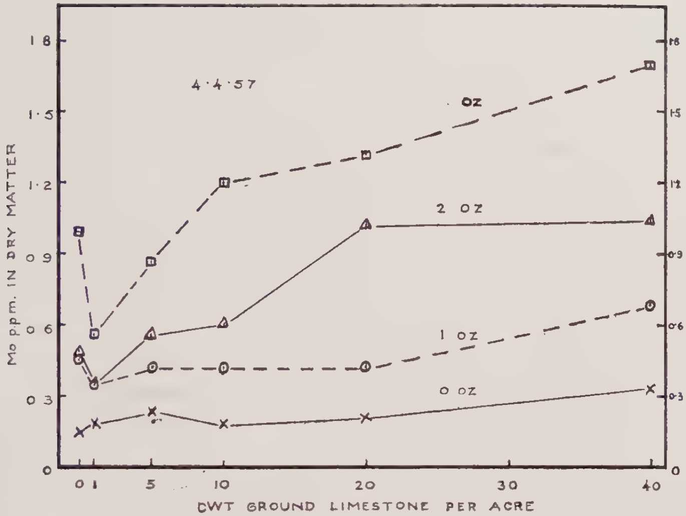


Fig. 15.—Molybdenum content of white-clover leaflets on 4 Apr. 1957.

As shown in Table 5 the manganese content of the white-clover leaflets obtained at the first sampling was very high, ranging from 387 p.p.m. down to 205 p.p.m. There is little influence on the figures from applications of molybdate, but use of limestone has, on the average, reduced the manganese content somewhat, without any worthwhile differences being shown between the heavier treatments. Subsequently however the influence of the limestone in reducing the manganese content becomes more important, even over-riding the seasonal variations. This is demonstrated by the average figures, omitting the data for the first set of samples, set out in Table 6.

It is clear from the data of Table 6 that for each molybdate treatment use of increasing amounts of ground limestone has consistently reduced the manganese content of the clover. It would seem too that this reduction has been more effective, although not markedly so, in the presence of the largest dressing of molybdate at the intermediate rates of liming. On the other hand, for a given level of application of molybdate, molybdenum increases with decrease in manganese content. This is probably an indirect effect from the known property of limestone dressings in raising the intake of molybdenum but in decreasing that of manganese because, when the data of the four samplings for a given rate of application of limestone are plotted, there is no relationship between the concentrations of these elements.

No relationship between manganese content and the nitrogen and calcium levels in the leaflets has been found.

TABLE 5. MANGANESE CONTENT (P.P.M. OF D.M.) OF WHITE-CLOVER LEAFLETS ON DIFFERENT DATES, FOLLOWING USE OF GROUND LIMESTONE AND SODIUM MOLYBDATE

Ground Limestone (cwt/acre)	Sodium Molybdate (oz/acre)	21.10.55	4.5.56	4.4.57	29.10.57
0	0	260	207	140	166
	1	219	208	164	161
	2	301	207	162	157
	4	387	220	162	143
1	0	268	—	174	134
	1	254	176	135	157
	2	245	202	175	136
	4	288	171	163	146
5	0	201	158	159	149
	1	260	163	155	151
	2	246	148	151	153
	4	249	131	135	145
10	0	250	154	139	122
	1	252	145	134	124
	2	279	139	147	123
	4	219	133	100	109
20	0	209	118	130	133
	1	205	123	119	116
	2	278	105	119	104
	4	245	90	115	112
40	0	250	128	100	78
	1	215	88	108	93
	2	228	117	94	99
	4	253	107	93	110

TABLE 6. AVERAGE VALUES OF MANGANESE CONTENT (P.P.M. OF D.M.) OF WHITE CLOVER LEAFLETS FOLLOWING DIFFERENTIAL LIMESTONE AND MOLYBDATE APPLICATIONS

Limestone (cwt./acre)	Sodium Molybdate (oz/acre)				
	0	1	2	4	Average
0	171	178	175	175	175
1	154	156	171	160	160
5	155	156	151	137	150
10	138	134	136	114	130
20	127	119	109	106	115
40	102	96	103	103	101
Average	141	140	141	132	138

DISCUSSION

From the analytical data for soil samples given in Tables 2 and 3 attention may be drawn to several matters of interest. By reason of the drift towards higher pH values in both the upper and lower sections of the soil it would seem that in the three and a half years since laying down of the pasture some changes are taking place which, independently of any effects of the ground limestone, are acting to reduce the acidity. There is also evidence in both sets of samples that the pH value in a given season is lower in the spring than in the autumn.

A change in soil conditions following use of limestone has resulted, as shown in Table 3, in an apparent decrease in the availability of phosphorus and potassium in the topsoil as measured by extraction with citric acid, and of potassium and magnesium as indicated by the exchangeable-base data. For potassium, the reduction has been demonstrated by two quite different types of chemical reagent. The reduction in solubility of the phosphorus has been relatively great, a reduction which it seems could hardly be due solely to the small increase in pH value of all except the most highly limed sample: indeed there is an indication that in this last sample phosphorus has increased in solubility. These data therefore suggest that use of even light applications of ground limestone have brought about some rather deep-seated change in the soil, which has as yet not penetrated into the 2–4 inch depth. Further investigation of this matter appears to be warranted.

This change in behaviour of the soil towards chemical reagents is moreover important from an advisory point of view. If very low analytical data are going to be obtained from treated soils then their interpretation is going to be very difficult, especially when, as in the case of this experimental pasture, growth of fodder and increased carrying capacity for stock have been so outstanding.

Examination of the analytical data, especially for molybdenum and manganese, shows that various relationships between the levels of these elements in the clover leaflets and the treatments applied are exhibited.

If the data for molybdenum content are graphed after expressing them in the form $\log_{10}$ (Mo in p.p.m.), while the rate of application of molybdate is given in the form $\log_{10}$ (oz + 1), then in a given set of samples straight lines can be drawn through the four points of the molybdate applications for each rate of application of limestone. A series of six approximately parallel lines is usually obtained for the samples of each date. In some cases one or two of the lines will not be parallel to the remainder of the set. Also the lines for different sets of samples are approximately parallel: for example the data for the samples of 4 April and 29 October 1957.

The data may also be graphed in a different way, plotting $\log_{10}$ (Mo in p.p.m.) against rate of use of limestone expressed as $\log_{10}$ (cwt + 1); in this case, for any given set of samples, figures for a constant rate of use of molybdate are taken. It is again found that the data fall on a straight line. In illustration of this the two following equations are given:

(1) for samples of 21 October 1955 representing treatments with 2 oz sodium molybdate per acre:

$$\log_{10} (\text{Mo p.p.m.}) = 0.490 \log_{10} (\text{cwt} + 1) - 0.942.$$

(2) for samples of 4 April 1957 when no molybdate was used:

$$\log_{10} (\text{Mo p.p.m.}) = 0.180 \log_{10} (\text{cwt} + 1) - 0.850.$$

To see how closely the determined figures and those figures calculated from the graphs agree, the following data are given:

Limestone (cwt/acre)	Example 1		Example 2	
	Detd.	Calcd.	Detd.	Calcd.
0	0.11	0.11	0.14	0.14
1	0.20	0.16	0.18	0.17
5	0.23	0.28	0.23	0.20
10	0.39	0.37	0.18	0.22
20	0.67	0.50	0.21	0.25
40	0.54	0.70	0.33	0.28

A further interesting relationship, of the same kind as the above for molybdenum, is found if the average figures for manganese in the right hand column of Table 6 are similarly plotted against rate of use of ground limestone. The equation found for the straight line through the points as plotted is:

$$\log_{10} (\text{Mn in p.p.m.}) = 2.260 - 0.145 \log_{10} (\text{cwt} + 1).$$

The satisfactory agreement between the original analytical data and those calculated from this equation is as follows:

Limestone (cwt/acre)	Manganese p.p.m.	
	Original	Calculated
0	175	182
1	160	164
5	150	140
10	130	128
20	115	117
40	101	107

The explanation of these logarithmic equations may be that the absorption of molybdenum or manganese is not directly proportional to the concentration of the amounts of available molybdenum or manganese but to their logarithms. It is interesting to note that other workers' data (Reisenauer 1956) not only for uptake of molybdenum by pasture, but also for other absorption data (Dick 1957) follow a logarithmic form similar to the above.

A further matter, which is of importance to the farmer, arises from a comparison of Fig. 2 with Fig. 4. The latter gives a view representative of the whole area later in its history; in other words, by visual estimation there was no difference in the amount of fodder on any one plot as compared with another. Thus, whatever differences due to treatment there may have been in the earlier period after establishment, later on all such differences had disappeared. Now, on these plots the only common treatment was the use of 5 cwt of superphosphate per acre. Was this sufficient therefore, although slower in action than superphosphate plus limestone and molybdate, to produce a very satisfactory pasture on the acid phosphate-deficient soil of the experimental area? It is hoped that this matter may be examined further.

There will still remain the question to be answered: which method, from the farmer's point of view, will be the most satisfactory economically?

ACKNOWLEDGEMENT

The authors wish to thank Messrs. L. Hodgson and K. J. Dodson for the soil data presented in Table 3. Mr. Dodson also gave valued assistance when the plots were cut to obtain the yield data for the pasture.

REFERENCES

DICK, A. T. 1957: *Proc. 7th int. Grassl. Congr. 1956*, p. 368.

REISENAUER, H. M. 1956: *Soil Sci. 81:* 237.

"CLOUD" OR VASCULAR BROWNING IN TOMATOES

IV. POLYPHENOL OXIDASE ACTIVITY IN CLOUD-SUSCEPTIBLE FRUIT

By ELSA B. KIDSON, Soil Bureau, Department of Scientific and Industrial Research, Seconded to Cawthron Institute, Nelson.

(*Received for publication, 26 June 1958*)

Summary

The browning in the vicinity of the vascular system characteristic of cloud appears to be polyphenol oxidase browning.

Polyphenol oxidase has been found to be active in abnormally ripening cloud-affected wall tissue, but appears to be inactive in normally ripening wall tissue even in cloud-affected fruit.

High polyphenol oxidase activity has been found in very young fruits, diminishing and finally disappearing with increasing maturity.

It is suggested that cloud-affected fruits show an abnormal retention of polyphenol oxidase activity in the blotched areas. This activity persists in cloud tissue ripened to the orange-red stage on the plant.

INTRODUCTION

"Cloud" in tomatoes, a form of uneven ripening, has been described in a previous paper of the series (Kidson and Stanton 1953a). It has been reported in many parts of New Zealand, particularly in glasshouse fruit. It appears to be identical with a form of blotchy ripening described by Bewley and White (1926) and with vascular browning as described by Conover (1949). It has been identified by the author in Britain where it is frequently of widespread occurrence. There is evidence that the same disorder causes serious loss in the Netherlands and in parts of North America.

Cloud is characterised by the appearance of green or brownish-green areas on the ripening fruit. The most shaded parts of cloud-susceptible fruits, i.e., parts touching the main stem or other fruits, are those most noticeably affected and are often the only areas so affected. Underlying the blotches there are dark veins following the vascular system. The browning appears to develop as the fruit approaches the colouring stage (Kidson and Stanton 1953c). Recent work has shown that the browning takes place in the parenchyma cells in the vicinity of the vascular strand rather than in the vascular tissue itself, observations agreeing with those of Seaton and Gray (1936) for blotchy ripening. The brown colour is usually associated with a collapse of the affected cells, in extreme cases resulting in the formation of channels through the flesh.

There are marked varietal differences in susceptibility to cloud. Stanton testing dwarf varieties on outdoor sterilised soil found that whereas one variety averaged 18 to 26 cloud fruits per plant, five others averaged less than 5 cloud fruits per plant and one variety was free

N.Z. J. agric. Res. 1: 896–902.

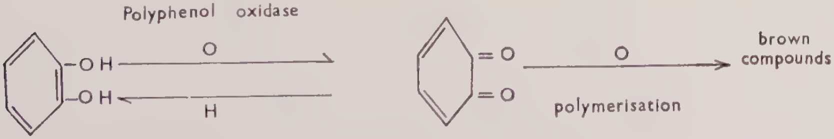
from the disorder (unpublished data, 1954). Massey and Winsor (1957) examined the incidence of blotchy ripening in ten varieties and found that Potentate was much the most susceptible, with 20.7% of affected approaches the colouring stage (Kidson and Stanton 1953c). Recent work by the author has produced further evidence of striking varietal differences.

Cloud is increased by soil sterilisation, heavy watering, and wet, cool seasons (Kidson and Stanton 1953a, b). It has been induced in water-culture experiments by conditions of low light and high humidity (Kidson 1956). It can be much reduced or prevented in soil-grown plants by liberal dressings of fertiliser during the season (Kidson and Stanton 1953a) and by a reduced water supply. In water-cultures under cloud-inducing conditions it has been almost eliminated by trebling the osmotic pressure of the nutrient solution either by increased quantities of major elements or by the addition of sodium sulphate (Kidson, unpublished data). Hall and Dennison (1955) have found that cool temperatures, shade, and mist increased vascular browning.

Percentage of dry-matter and of total carbohydrate appear to be below normal in cloud-affected fruit (Kidson and Stanton 1953b, c), and treatments reducing cloud have raised the percentage of these constituents in the fruit. Massey and Winsor (1956, 1957) found blotchy ripening to be associated with a reduced percentage of total solids, reducing sugars, acids, and nitrogenous compounds. Cloud-affected tissue has been found by the author to have a lowered acid content, as measured by titratable acidity.

The Function of Polyphenol Oxidase in the Respiration of Tomato Fruits

It is known that most browning of fruits is produced by the action of polyphenol oxidase. Polyphenols with ortho di-phenolic groups are oxidised by this enzyme to corresponding quinones. The browning takes place by what is referred to by MacDougal and Dufrenoy (1946) as the decompensated action of polyphenol oxidase. Normally the oxidation is reversed by some reducing system, but in its absence, brown compounds are formed as a result of further oxidation and polymerisation. The oxidation products of the quinones may unite with constituents of the cytoplasm (MacDougal and Dufrenoy 1946).



The browning usually occurs as a result of injury by mechanical damage or invasion by a pathogen. In fruits such as apple, pear, or quince, any damage to the flesh will result in a rapid appearance of polyphenol oxidase browning. Normal tomatoes do not behave in this fashion, at least at the ripening stage. There is no rapid development of brown colour with mechanical damage. If the enzyme is present, the system is protected from complete oxidation and browning.

It appears that polyphenol oxidase may function as a respiratory enzyme in many but not all plant tissues (Bonner and Galston 1955). The polyphenol-quinone oxidase system may act in a chain of reactions transferring electrons from certain respiratory substrates to the oxygen of the air (W. D. Bonner 1956), or in the last step as a terminal oxidase (Bonner and Galston 1955). Observations by W. D. Bonner (1955) with mushrooms and mung beans have suggested that the enzyme functions anaerobically in respiration. He concludes that polyphenol oxidase reacts directly with oxygen only under conditions of high oxygen tension and that under normal conditions it acts as an electron transport system using cytochrome C as an acceptor.

The function of polyphenol oxidase in the respiratory system of the tomato fruit has been investigated by Marh and Feljdman (1956). They suggested that in the two different varieties tested, the part played by polyphenol oxidase in oxygen absorption in the oxidising systems of tomato fruits was very considerable (more than 50% of the total respiration).

Work reported in the present paper has confirmed the presence of this enzyme in local tomato varieties and has indicated differences in polyphenol oxidase activity between healthy and blotched areas in cloud-affected tomatoes.

Polyphenol Oxidase Activity in Cloud-affected and Healthy Wall Tissue

Blotched and normally ripening areas in fruits of the variety Potentate have been compared for their ability to oxidise catechol to the brown stage. The fruit wall plus skin was disintegrated in a blendor with an equal weight of water. The disintegrated material was filtered through plastic gauze to remove the coarsest particles and to the suspension passing the filter was added a solution of catechol. It was found that cloud-affected (blotched) tissue, i.e. tissue from the areas showing abnormal ripening and brown veins, invariably produced a brown colour with catechol indicating polyphenol oxidase activity. The test was repeated many times, and in no case did samples of normally ripening, cloud-free areas of cloud-affected fruits give this brown colour with catechol, nor did the reaction take place in similarly treated walls of healthy ripening fruits. If slices of blotched tissue were treated with catechol after the removal of the brown veins many cells not formerly affected developed the brown colour. Unlike the fruit walls, ground seeds of both healthy and cloud-affected fruits gave a strong reaction with catechol, most marked in the seed coat.

Site of Polyphenol Oxidase Activity

It was found that browning originated in the solid particles of disintegrated tissue and that the activity of the blotched suspension was much reduced by filtering methods which removed a large proportion of the insoluble material. Slices of blotched tissue treated with catechol developed a widespread browning which appeared to be strongest where the chloroplasts were most numerous and, in many large parenchyma cells, appeared to be confined to the vicinity of the nucleus where most or all of the chloroplasts were concentrated. The usual distribution of browning was heavy in and near the vascular bundles, less concentrated in the larger parenchyma cells and occasionally fairly heavy in cells near the inner surface of the fruit wall.

Inhibiting Effect of Extracts of Healthy Tissue

Healthy fruit-wall tissue had an inhibiting action on the polyphenol oxidase browning of disintegrated cloud tissue. If a large enough proportion of healthy suspension was added, no browning occurred. Smaller proportions prevented the full development of the colour. Wall tissue of fruits from cloud-free treatments was more effective in this way than that of healthy areas of cloud fruit. The inhibitory action appeared to be present in the water-soluble fraction, a clear filtered solution retaining its effectiveness.

Effect of Washing

It was found that the treatment of slices of blotchy tissue with certain solvents greatly accelerated the speed and extent of the catechol browning. This effect was shown by slices washed with 80% acetone, with 95% ethyl alcohol, both followed by water, or by water alone. The acetone had the most pronounced effect and the water alone the least effect. In contrast with cloud tissue which browned heavily, slices of wall of normally ripening areas of cloud fruit gave only slight traces of browning or no browning at all with catechol after treatment with these solvents. As might be expected, such treatment resulted in loss of organic acids as measured by titratable acidity.

Effect of Organic Acids and Glucose

Ascorbic acid was very effective in preventing browning in suspensions of blotched tissue, and its action was related apparently more to its reducing power than its effect on pH. There was evidence that citric acid and malic acid had some inhibiting effect. At low concentrations where pH was not a factor, the inhibiting action of citric and malic acids was slower and less effective than that of equivalent amounts of ascorbic acid. Glucose inhibited the browning if present in sufficient quantity. The addition of this sugar increased the acidity of the suspension.

Effect of pH and of Buffer Solutions

Attempts to determine the effect of pH on the polyphenol oxidase browning were complicated by apparent specific effects of certain buffers. With a phosphate-citrate buffer of pH 3.70 to 4.70 the browning of disintegrated blotched tissue did not occur below 4.25 and increased with rising pH. Healthy areas of cloud fruit gave no brown colour over this range. Ground seeds treated with a phosphate-citrate buffer did not change colour below pH 4.80. Tests on disintegrated cloud tissue from fruits of the variety Pearl Harbour with a phosphate-citrate buffer, an acetate buffer and a buffer of phosphate alone, all at a pH of approximately 5.0, showed a strong polyphenol oxidase browning with the acetate buffer and a trace only with the phosphate-citrate and phosphate buffers. The colour with the acetate buffer developed faster and was stronger than that with water alone at a similar pH. This action of acetate buffer was observed also with disintegrated seeds. With a phosphate-citrate buffer in the pH range of 4.45 to 6.45 no catechol browning of seed particles took place below 4.80 and above that figure there was slight reaction increasing with rising pH. With an acetate buffer of pH 4.25 to 5.65, on the other hand, there was a strong reaction over the whole pH range. Seed particles with acetate at a pH of 5.0 gave a darker catechol browning than with water, even though the pH of the water suspension was as high as 6.50.

The tests indicated that the citrate-phosphate and phosphate buffers inhibited the browning to a large extent, a reaction which may be related to an ability to form complexes with copper, the metal activator of polyphenol oxidase. Acetate buffer had the opposite effect.

Whereas acetate-buffered cloud-affected tissue and seeds produced a strong oxidation of catechol to the brown stage, wall tissue from normally ripening areas of cloud fruits did not brown catechol in the presence of acetate, even when the tissue had previously been washed with acetone and water.

Age of Fruit and Polyphenol Oxidase Activity

It has been noticed that very young tomato fruits darken easily on bruising or cutting, and that this effect is much reduced or absent in more mature fruits. Tests with tomatoes at different stages of development showed that the skin and vascular systems of the walls of very young fruits had a high polyphenol oxidase activity as measured by the speed and extent of the development of brown colour in acetate buffer on the addition of catechol. The reaction diminished with increasing maturity and was not shown by normal fruits at the colouring stage. The same general trend of decreasing polyphenol oxidase activity with increasing maturity as measured by this method was shown by a number of different varieties, some of them grown outdoors in conditions not conducive to cloud.

DISCUSSION

The tests have shown the presence of polyphenol oxidase in abnormally ripening areas of cloud fruit and suggest that the browning is a polyphenol oxidase browning. So far it has not been possible to demonstrate that this enzyme is active in the wall tissue of ripening fruits not affected by the disorder. Moreover, normally ripening wall areas of cloud fruit have shown no polyphenol oxidase activity except for an occasional slight trace. It is suggested that, in healthy fruits, polyphenol oxidase is not present or is inactivated at the onset of ripening, and its occurrence in cloud-affected areas is an abnormality. Very young fruits had a high polyphenol oxidase activity as measured by the rapidity with which they oxidised catechol to the brown stage. This activity was found to diminish and finally disappear with increasing maturity. A drop in the activity of polyphenol oxidase as a respiratory enzyme with increasing age of the fruit has been found by

Marh and Feljdman (1956) for two different varieties tested. With increasing maturity there was a continuous fall in the intensity of respiration including that controlled by polyphenol oxidase. This was accompanied by a decrease in the fraction of respiration catalysed by metal-containing enzymes (including polyphenol oxidase) and an increase in that catalysed by the flavin enzymes. It appears that in cloud-affected fruits the blotched areas retain a juvenile characteristic of high polyphenol oxidase activity. That this is not merely an indication of unripe tissue is shown by the fact that the power to brown catechol is retained by cloud-affected tissue ripened on the plant to the orange-red stage.

Not only is the enzyme retained in the blotched tissue, but the oxidising action is inadequently compensated by a reducing mechanism, at least in certain cells. The inhibiting action *in vitro* of citric and malic acids, both acids of the Krebs cycle, and the increase in browning produced by acetate are of interest from the point of view of the dehydrogenase systems which may function in this respect. Acetate has been found by Laties (1949) to have a strong inhibitory action on respiration, probably by competition with one of the first intermediates in the oxidation of pyruvate. Ascorbic acid has been very effective in preventing the browning *in vitro* but whether it functions in this way in the plant is in some doubt. Appreciable quantities of ascorbic acid (10–15 mg per 100 g of fresh tissue) have been found in cloud-affected patches and also of total organic acids as measured by titratable acidity, though both tend to be lower in blotched than in healthy areas of the same fruit. No examination has been made of the organic acid content of the cells in the immediate vicinity of the browning and these may possibly show greater differences from healthy tissue. Whatever the direct cause of the browning, environmental conditions that tend to produce it, viz. low light, high humidity, high available water, are those that tend to reduce the content of sugars and their derivatives either by reduced photosynthesis or increased water uptake.

ACKNOWLEDGEMENTS

The author wishes to thank Professor V. W. MacFarlane of the University of Queensland for suggesting the line of work, Dr K. Strzemienski of the Soil Bureau for much help and advice, Miss Anne Hole for conscientious and able assistance with water-cultures and laboratory work, Mr R. L. Closs of the Dominion Physical Laboratory for the provision of a humidity control apparatus, and Mr D. J. Stanton without whose willing co-operation this investigation would have been seriously handicapped.

REFERENCES

BEWLEY, W. F.; WHITE, H. L. 1926: *Ann. appl. Biol. 13:* 323.

BONNER, J.; GALSTON, A. W. 1955: "Principles of Plant Physiology". Freeman, San Francisco.

BONNER, W. D. 1955: *Plant Physiol. Proc. Suppl. 30:* xxx.

— ——1956: *Ibid. 31:* xli.

CONOVER, R. A. 1949: *Plant Dis. Reptr 33:* 283.

HALL, C. B.; DENNISON, R. A. 1955: *Proc. Amer. Soc. hort. Sci. 65:* 353.

KIDSON, E. B. 1956: *Cawthron Inst. annu. Rep. 1955–56.* p. 48.

KIDSON, E. B.; STANTON, D. J. 1953a: *N.Z. J. Sci. Tech. A34:* 521.

——————1953b: *Ibid. A35:* 1.

——————1953c: *Ibid. A35:* 368.

LATIES, G. G. 1949: *Arch. Biochem. 20:* 284.

MACDOUGAL, D. T.; DUFRENOY, JEAN 1946: *Plant Physiol. 21:* 1.

MARH, A. T.; FELDJMAN, A. L. 1956: *Biokhimija 21:* 33.

MASSEY, D. M.; WINSOR, G. W. 1956: *Rep. exp. Res. Sta. Cheshunt,* 1954 p. 50.

——————1957: *Glasshouse Crops Res. Inst. annu. Rep. 1954–55.*

SEATON, H. L.; GRAY, G. F. 1936: *J. agric. Res. 52:* 217.

THE DEVELOPMENT OF AN ELECTRONIC APHID-COUNTER

By A. D. LOWE* and W. V. DROMGOOLE†

I. THE PROBLEM OF COUNTING APHIDS

A. D. LOWE

(*Received for publication, 18 June 1958*)

Summary

Various methods of estimating aphid populations are discussed and their limitations are noted.

Details are given of the construction and operation of an electronic counter by means of which normal colonies of aphids can be counted with a repeatability of ± 3%. It is essentially an instrument for use in the laboratory, where a stable power supply is normally available.

INTRODUCTION

The problem of aphids, especially the cabbage aphid (*Brevicoryne brassicae* L.) on cruciferous crops in Canterbury, and their associated virus diseases, is one of long standing. These crops, either as forage or seed crops, are present in some form throughout the year. Chou moellier, kale, rape, swedes, turnips, and mustard are used extensively as feed from early December to August, and chou moellier and kale are regularly grown for seed north of the Rangitata River, and rape, swedes, and turnips in areas south of this boundary.

Seed crops are in the ground for periods of from eight to twelve months, and feed crops for periods ranging from three months for rape up to ten months for swedes and chou moellier.

The first approach to this problem was to endeavour to find a satisfactory chemical control to give some relief to growers. This was not only envisaged as an end in itself, but was an opportunity to examine the field populations and thus determine sound lines along which long-term work might proceed. The project was mooted in November 1954, and that summer gave opportunity for preliminary exploration. The next season saw chemical control work on an organised basis, and it was quickly evident that the chief difficulty was in the judging of results. Since a long-term approach through a study of population dynamics was by this time envisaged, considerable attention was paid to the question of sampling and counting aphids.

It will be evident from the above outline of the problem that various crops, having differing foliage systems are involved. These differences are intensified under conditions of autumn drought, when the foliage systems of swedes and turnips tend to be rather sparse as

---

* Entomology Division, Department of Scientific and Industrial Research, Ashburton.

† Industrial Development Department, University of Canterbury.

N.Z. J. agric. Res. 1: 903–912.

compared with conditions under irrigation or adequate rainfall. The presence of virus diseases has very distinct effects in foliage reduction in some varieties of these crops, and further complicates the position.

It was also evident that for this aspect of the problem, only limited manpower would be available. It appeared likely that any sampling system in regular weekly use would be able to employ not more than two officers, with a third available occasionally, but not regularly, because of other duties.

SAMPLING TECHNIQUES

An examination was therefore made of those sampling systems which had been devised for use with small insects, to see if any of these would contribute to the solution of this particular problem. The counting of arthropod pests of small physical dimensions has always been recognised as a problem beset with particular difficulties. Not only do many such pests develop prodigious numbers under field conditions, but actually seeing, counting, and recording the individuals introduces problems in human fatigue which, in cases of limited manpower where a few individuals do all the sampling and counting, seem to be insurmountable. In this particular problem the following systems were examined and tested:

The *brushing machine* technique of Henderson and McBurnie (1943) which has been used with much success against mites did not appear to be useful since hand-brushing showed that to dislodge an attached aphid required a pressure which in a large proportion of the population ruptured the body.

The *imprinting* technique of Venables and Dennys (1941) appears quite suitable for aphid populations when they are dispersed on the leaves, as after a flight, but the cabbage aphid develops huge colonies numbering many hundreds, all packed closely together on leaves, and also develops dense colonies in the growing points of the plants. Further, in bad cases of infestation, leaves become curled and distorted and enclose large numbers of aphids within the folds. These facts preclude using this system for this insect.

Systems currently used for the sampling of aphids which were examined and tested were the leaf-sampling system (e.g. Church and Strickland 1954) and the ranking system, by which plants are classified into several categories, and some attempt made to establish the levels of infestation thus represented. Both these systems have been critically examined, and their limitations explained by Strickland (comp., 1957), in a report on cabbage aphid assessment on Brussels sprouts in England and Wales over a ten-year period.

The system of aliquot sampling as used by Banks (1954) in counting the bean aphid was also examined and tested.

Not one of these systems appeared to offer very much hope of development of a sampling system of the high standard required for the project being undertaken. Nor did any system of visual counting in the field have any application, since it was evident from total plant counts that had been made on both sprayed and untreated plants taken at random (Table 1) in a field of poorly foliaged swedes ten weeks old, that the numbers of aphids present were likely to exceed 5,000 per plant at times, and it was suspected that the worst cases of infestation in well foliaged plants probably involved populations up to 20,000 aphids per plant.

TABLE 1. Aphids per Plant on Swedes 24.1.56

Sprayed	Not Sprayed
2424	4791
107	6563
2103	5973
13	6135
360	5882
1451	2065

It was hoped to develop a system of sampling and counting which would not err by more than ± 5%. The error of systems examined varied from that given by Banks (1954) with a maximum of ± 24% for certain classes of samples to that outlined by Strickland (1957) in which four classes of rankings were used. When translated to "rounded off weighting values", with twelve observers available and peak densities averaging 1,531 aphids per plant over three years, and after statistical manipulation, errors varied from 7.7% to 1.1% for different ranks. The latter author states that other data were available for larger numbers of plants and "these results show that the standard error per plant is near 22.6%".

Strickland (1957) also cites figures from the testing of the method of experienced observers attempting to estimate the actual numbers of aphids on a plant, when errors varying from 7% to 500% were recorded, 3,000 aphids and 20,000 aphids both being estimated as 3,250.

It will readily be seen that these authors accepted a much higher error than that aimed at in this work, in which fairly high numbers of aphids per plant were expected, and there was a definite limitation on available manpower. The essential criteria of any sampling system would be a high degree of accuracy, economy in man-hours, and, in view of the many varieties of cruciferous plants involved in the problem, applicability to the various types of plants likely to be encountered. While the system would be useful in such short-term projects as the application of chemicals, it would be chiefly required in the long-term studies of population trends. Low degree of error was important since it was hoped that sampling would give an accurate picture of both the population growth and distribution curves of this pest, yielding valuable information on its field biology. Such fundamental information would help associated workers to make a critical review of the parasite-predator complex, and the importance of insect diseases. Correlations were to be sought against climatic conditions, and it was hoped that sufficient information having a high degree of accuracy might be collected to enable some contribution to be made to the theory of insect population dynamics. It was important therefore that figures collected should be comparable not only from crop to crop, but also place to place and season to season. It has been stated elsewhere (Lowe 1958b) that this aphid can theoretically double its population in 24 hours in the absence of natural enemies, and it has been shown that it does in fact double its field numbers in 10 days during summer. It was essential therefore that samples be collected and the insects killed for counting within a reasonably short period, before the high rate of parthenogenetic reproduction could substantially alter the population level.

In view of all these circumstances, the whole plant was chosen as the sampling unit, and an endeavour was made to count the individuals. This system continued during the spring of 1955 (Lowe 1958a), when a chou moellier seed crop was thoroughly examined by weekly or two-weekly counts from 21 September to 15 November, when populations reached an average of 640 aphids per plant. In 1956, a similar trend was evident. To establish these figures, together with wider examination of further samples in order to gain a picture of the distribution curve, as many as 105 samples were drawn and counted in one day of 12 hours by three officers. Such counting was however only possible when populations averaged about 350 aphids per plant. Normally, only 2 officers were used, each taking and counting 12 samples per 8-hour day. Statistical examination showed that this range of samples was insufficient to give the required level of accuracy when tested. It was also evident that fatigue severely taxed the officers concerned when 12 large samples had to be collected and counted in one day. It was decided that some method of mechanical counting should be investigated, and the problem was taken to the Canterbury College Industrial Development Department with the suggestion that either the aphids might be counted while passing a light beam, or that at the least a hand-operated switch activating a ticking metronome might reduce the fatigue involved in making visual counts.

USE OF THE ELECTRONIC COUNTER

After some preliminary trials in which the density of the aphids appeared to be insufficient to justify further work with the light-beam suggestion, the Department devised an electronic counter (Fig. 1) depending essentially on the alteration of resistance between two small electrodes inserted in a tapered glass tube, and a full account of the construction and circuitry of this instrument is given by Dromgoole in Part II of this paper.

It remains to give an account here of the method of preparation of the sample and the accessories associated with its use. It was agreed that the Department would be responsible only for the electronic instrument, and that the method to be employed in preparing the sample and feeding it in suspension form through the electrode tube would be primarily the responsibility of the Entomology Division. However, for the purpose of trials and preliminary calibration the Department had prepared a 600-ml beaker by attaching a glass tube

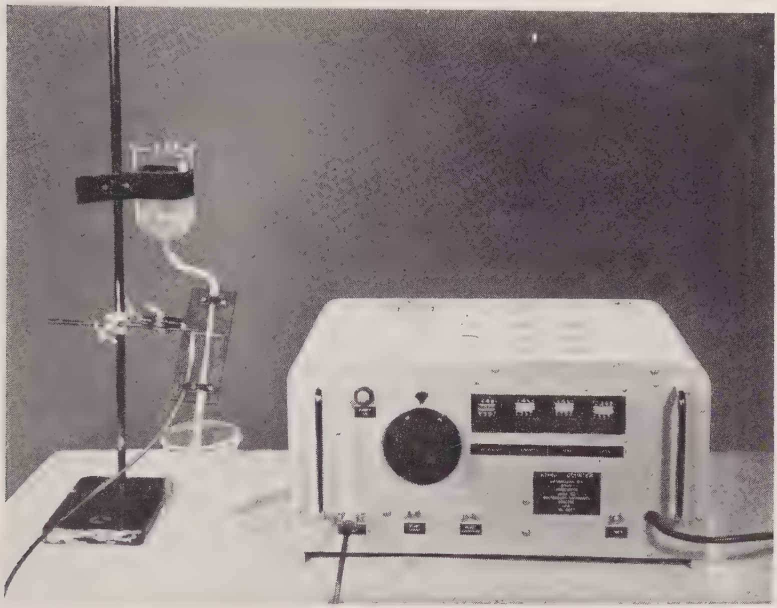


Fig. 1. *Left*: Aphid-receiving beaker and electrodes. *Right*: Electronic counter.

to its base. When this was mounted on a suitable retort stand and attached by rubber tube to the electrode, mounted lower on the same stand, it worked very satisfactorily and has, in spite of a number of attempted improvements, remained the most accurate method tried.

The sample is prepared by washing the aphids from the leaves with water fed from a mixer tap at a temperature of 70°–75°c on to an organdie filter stretched over a glass funnel. This temperature kills the aphids and removes their waxy coating, while higher temperatures cause them to cling together in clusters, unsuitable for counting. They are then transferred to a beaker of tap water, stirred lightly to make an even suspension, and poured through the electrode. During the counting, the suspension is kept stirred lightly in the beaker, but not so strongly as to make a vortex, as this causes air bubbles to pass the electrode giving spurious counts. The count may easily be checked by repeating the process.

A sensitivity dial is provided on the front of the instrument and it has been found in practice that a normal colony of aphids consisting of several sizes of apterous forms and some alates can be counted with a repeatability of $^{+}$ 3% if reasonable care is exercised in the technique used. Some spurious counts are recorded at the beginning and end of each run, when the water commences to flow, and ends its flow, over the electrode. These may be eliminated by the use of the "Start Count" switch or allowed for since they are a constant factor in each count made. It is evident that different sizes of aphids may be counted, so long as they can pass the electrode, by using the sensitivity switch provided and making a few trial runs.

The instrument could probably have an extended field of usefulness in counting many types of insects, or other small animals or particles provided a method could be devised of feeding these past the electrode in single file.

In this particular work it has been found advisable to keep the whole system of preparing the samples as simple as possible. This is the reason for the use of tap water as the medium of suspension. Though other media, such as alcohol and added detergents, have been tried, they have given no spectacular improvement in results.

A further improvement and extended usefulness may be possible with machined electrodes of specific sizes.

The instrument is essentially a laboratory tool, and we are informed by the Canterbury College Industrial Development Department that it requires a very stable power supply, which may limit its use in the field.

II. CONSTRUCTION OF THE COUNTER

W. V. Dromgoole

The unit was designed and constructed by the Industrial Development Department, Canterbury University College, and is described in detail.

The need for such an instrument was brought to our notice by Mr. Lowe of the Entomology Division of the Department of Scientific and Industrial Research at Ashburton. The problem was one of counting rapidly with reasonable accuracy the aphids as found on a particular plant leaf. The method then in operation was to inspect visually a small section of aphids uniformly distributed in a petri dish. Consideration was given to utilising the density change scanning the solution in the petri dish by means of a photo-electric cell. No experimental work was done on this apparatus as it was felt that an impulse counting method would give much higher accuracy.

The first method tried was similar to the final method adopted, but the electronic pulses were obtained by means of a light beam passing through a glass tube on to a photo-electric cell. Certain optical difficulties were experienced with the scattering of the light and it was found necessary to use a small machined slit in a brass body covered on each side by microscope glasses. Whilst this method produced satisfactory pulses as the aphids went past, it was found to be subject to blocking and it was at this stage that the electrode method was adopted.

The Electrode Tube

Use is made of the change in resistance in the liquid path between two small platinum electrodes inserted diametrically in the tapered section of a 3 mm glass tube (Fig. 2).

The tapered constriction is narrowest at the point where the electrodes are situated and the taper expands smoothly and longitudinally away from this point. This construction serves two purposes.

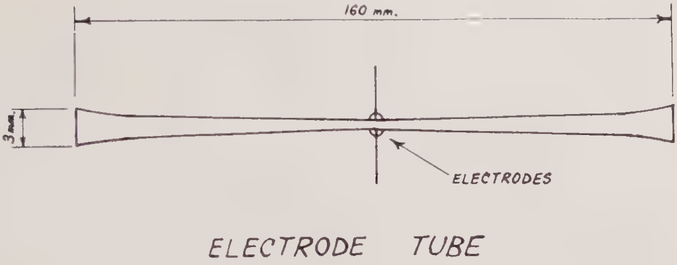


Fig. 2.—The electrode tube.

(a) The aphids sort themselves out in the upper section of the tube and tend to a single file when the electrodes have been reached. (b) The smooth taper away from the electrodes in the lower section of the tube reduces possibility of the set up of turbulence near or at the electrodes. It is imperative that the water flow near the electrodes be smooth and without air bubbles, since any disturbance of this nature will register as counts.

The glass tube is 16 mm in length, of 3 mm bore at its extremities, and the electrodes are situated approximately half-way along the tube. The distance between the electrode ends is about 2.5 mm. The insertion of the electrodes must be done by a competent glass blower, as the two platinum wires must make contact with the water, but must not protrude into the water, where they can cause eddies.

Any excessive protrusion may be removed with carborundum powder. The electrodes should be cleaned before use with a little Brasso or other mild abrasive, by placing a little in the tube and using a piece of PVC-covered wire of suitable diameter, or spurious signals may originate from some form of electrolysis. This condition, may, however, be easily checked by pouring water only through the tube with the dial set at zero, and observing the counter.

Circuit (Fig. 3) and Block Diagram (Fig. 4)

A voltage is connected with the + 300 volt H.T. supply via a start-count switch, and a 470 K res. to one electrode; the other one of which is at ground potential. There therefore exists at point X a D.C. voltage with respect to ground which is dependent on the resistance of the liquid path between the two electrodes.

Any change in this path produces a change of resistance, which is reflected as a voltage change across C1, in the form of a small voltage pulse as an aphid passes the electrodes. An examination of this pulse at point Y shows it to be both positive and negative sided, and usually there is some small amount of "water hiss" of a much higher frequency. This latter is reduced by Filter 1.

The pulse is amplified by V1, and both it and other irregularities of the zero base line of the waveform are treated by a low-level discriminator V2, which passes only the positive peak of the pulse, and

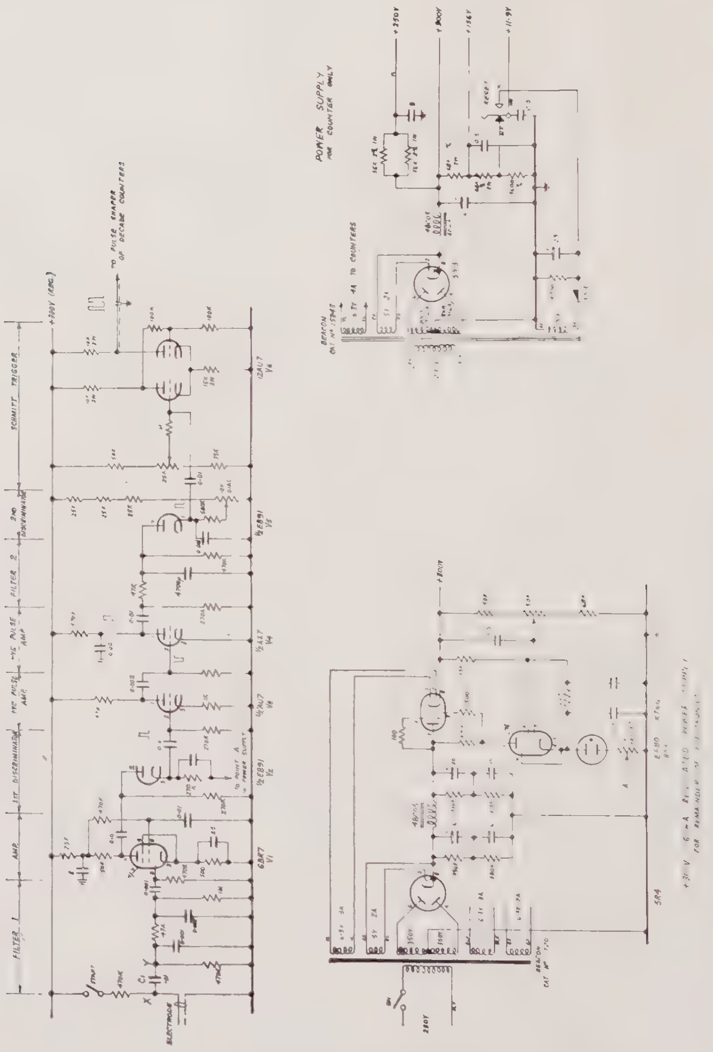

FILTER 1
AMP.
1ST DISCRIMINATOR
+VE PULSE AMP.
-VE PULSE AMP.
FILTER 2
2ND DISCRIMINATOR
SCHMITT TRIGGER
+300V (REG.)
TO PULSE SHAPER OF DECADE COUNTERS
ELECTRODE
START
6BR7 V1
½EB91 V2
½EB91 V5
12AU7 V6
TO POINT A IN POWER SUPPLY
POWER SUPPLY FOR COUNTER ONLY
+250V
+300V
+156V
+11.9V
BEACON
5R4
230V

Fig. 3.—Circuit diagram.

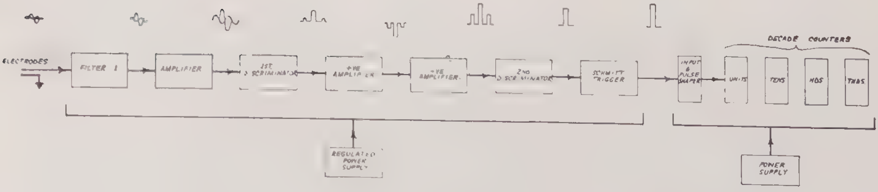

ELECTRODES
FILTER 1
AMPLIFIER
1ST DISCRIMINATOR
+VE AMPLIFIER
-VE AMPLIFIER
2ND DISCRIMINATOR
SCHMITT TRIGGER
INPUT & PULSE SHAPER
DECADE COUNTERS
UNITS
TENS
HDS
THDS
REGULATED POWER SUPPLY
POWER SUPPLY

Fig. 4.—Block diagram.

the level at which it passes this pulse is preset at about 2 volts (inside chassis).

This positive pulse is again amplified by V3, and inverted. Any hum or low-frequency component is severely attenuated by C2. A negative going pulse is therefore applied to V4, and again amplified and inverted to produce a large positive pulse at V4 anode, where it is passed (with others) to Filter 2, and a second discriminator V5, where the actual level required to operate the Schmitt trigger is selected on an aphid-sized basis.

The Schmitt trigger, V6, produces 60-volt pulses suitable to drive the pulse shaper in the decade counter.

The decade counter comprises:—

(a) A pulse shaper and input stage.

(b) 4 decade counters.

These are standard Philips units and are readily available. The display is such that the thousands, hundreds, tens, and units are read left to right on the unit. The counters may be immediately reset to zero at any time by the reset-counters switch.

The unit incorporates two power supplies, one a regulated type which supplies the above described circuit, and the other a simple unregulated one which supplies the decade-counter equipment.

In general the procedure is to pour the water and aphids into the beaker connected to the electrode tube, and then operate the start-count switch: then, as the last of the aphids disappear past the electrodes, release the start-count switch and read the decade counters. Finally operate the reset-counters switch to restore all counters to 0.

It has been found advisable to stir the water lightly as the count proceeds, to prevent aphids sticking to the side of the beaker, and also to prevent their adhering together near the bottom of the beaker as the count is approaching the end.

Accuracy

There has been a certain amount of difficulty in obtaining a large sample of aphids accurately counted for calibration. With a given sample of nominally 1000 aphids it is possible to repeat counts to better than ± 5% but we feel that with practice in mixing and pouring technique the accuracy will be improved.

It would appear that if some small quantity of water was run through the system while a count was in progress, much of this trouble would be obviated. The mechanics of this aspect of the problem are, however, not part of this section of the work.

ACKNOWLEDGEMENTS

The author is indebted to the Director of the Department, Mr. T. R. Pollard, for his enthusiastic interest, and to Mr. F. Downing of the

Chemistry Department of Canterbury College for his fine piece of glass-work.

REFERENCES

BANKS, C. J. 1954: A Method for Estimating Populations of *Aphis fabae* Scop. *Bull. ent. Res. 45:* 751-6.

CHURCH, B. M.; STRICKLAND, A. H. 1954: Sampling Cabbage Aphid Populations on Brussels Sprouts. *Plant Pathol. 3:* 76-80.

HENDERSON, C. F.; MCBURNIE, H. V. 1943: Sampling Technique for Determining Populations of Citrus Red Mite and its Predators. *U.S. Dep. Agric. Res. Circ. 671.*

LOWE, A. D. 1958a: Effect of Metasystox on the Cabbage Aphid. *N.Z. J. agric. Res. 1:* 37-43.

——— 1958b: Insect Pests of Brassicas. *Proc. Farmers' Conf. Lincoln.*

STRICKLAND, A. H. (Comp.) 1957: Cabbage Aphid Assessment and Damage in England and Wales, 1946-55. *Plant Pathol. 6:* 1-9.

VENABLES, E. P.; DENNYS, A. A. 1941: A New Method of Counting Orchard Mites. *J. econ. Ent. 34:* 324.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

I. INTRODUCTION, SOILS AND VEGETATION

By R. H. Thornton, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 19 June 1958*)

Summary

An introduction is given to a series of biological investigations of three tussock-grassland soils.

Profile descriptions and chemical analyses of the soils are given together with a description of the vegetation on the sites.

Introduction

In the extensive literature relating to the microbiology of soils there are relatively few references to the microbiology of grassland soils. In particular, little work appears to have been done on pasture grassland soils. New Zealand offers most favourable material for such studies, as large areas of grassland occur naturally and the all-important pasture grasslands have been, and are being, developed from former forest or natural grasslands, where progressive changes may readily be observed. In the investigations to be described, three soils, at present under naturally occurring low-tussock grassland, were examined. As the microbial populations of these perennial-grassland soils might be expected to have reached equilibrium, results could provide a guide and serve as a basis for future studies of the microbiology of soils, particularly those under introduced grassland.

The specific aims of the investigations were, firstly, to examine qualitatively and quantitatively the distribution in these soils of the following groups of organisms: fungi, yeasts, bacteria, protozoa, streptomyces, algae, and earthworms; secondly, to follow the seasonal changes in these populations; and thirdly, to correlate the differences in the distribution of the organisms with ecological factors. The present series of articles embraces the results of this work.

A further series of investigations has been carried out on two former tussock-grassland soils, cultivated, and sown to root crops and pasture grasses. Results of these investigations will be presented in a later series of articles, after which an attempt will be made, in a further article, to integrate the results of these two series of investigations. The soil population will be viewed as a whole to discover the important factors influencing populations and their interrelationships. It will be shown that the distinct microfloral patterns associated with these grassland soils are greatly influenced by the root systems of the higher plants, whereas the microfaunal populations are not so influenced. Physiological activities of the various groups of organisms will be considered in relation to their distribution in soil. This

N.Z. J. agric. Res. 1: 913–921.

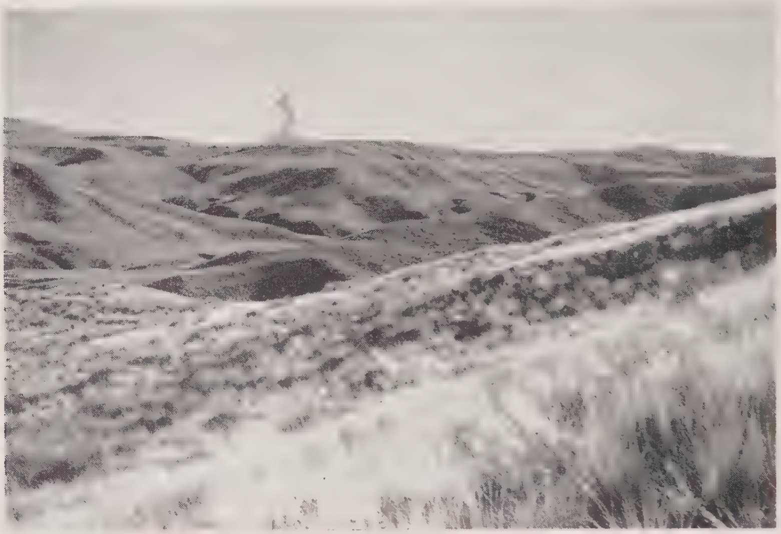


Fig. 1.—Site near Waiouru (on Taupo hill soil). Mt. Ngauruhoe in eruption in the background.

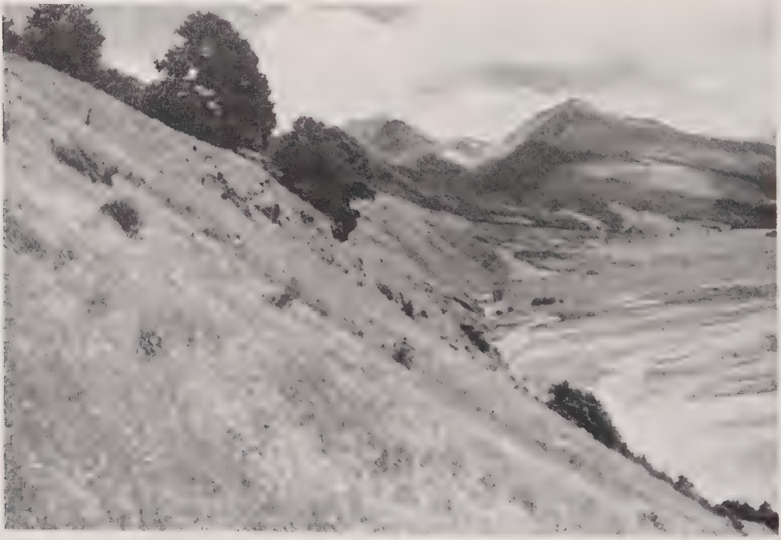
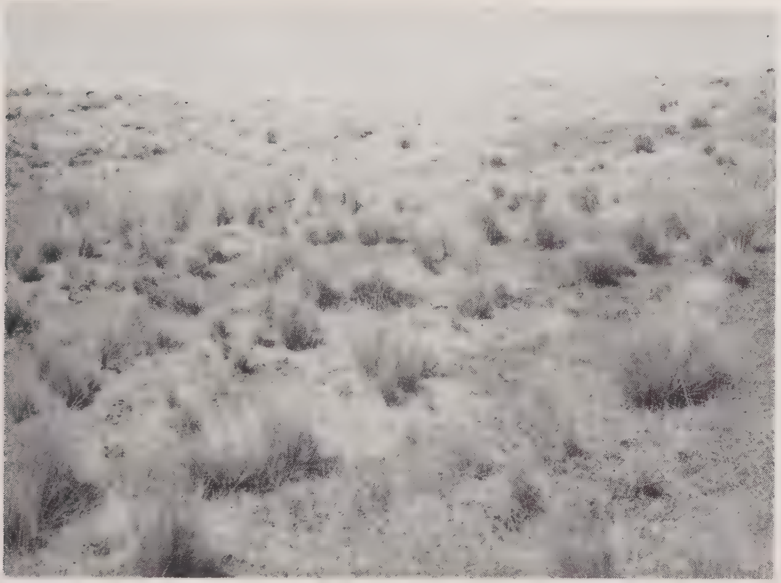


Fig. 2.—Site near Bealey (on Tekoa steepland soil).

appraisal of the co-operative studies by a number of workers, it is hoped, will lead to a better understanding of biological activities in soil.

In the following an account is given of the sampling method, employed, and of the soils and vegetations at the three sites selected. Results of investigations on particular groups of organisms are given in other articles in this series.



—Photo: A. P. Druce

Fig. 3.—Site near Alexandra (on Omarama steepland soil, moderately sloping variant).

SITES

The three soils employed in the present investigations were selected to represent a range of soils and climatic conditions under which present-day low-tussock grasslands occur. The selection of sites was governed by road accessibility, but even so winter samples could not be taken at the three sites. At each site as many samples as could adequately be examined, were taken.

Three localities were selected for the investigations, one in central North Island near Waiouru (map. ref., 1-mile sheet N122. 217437) and two in South Island, one on Black Range near Bealey (map. ref., 4-mile sheet S25 018018) and the other on Old Man Range near Alexandra in Central Otago (map ref., 4-mile sheet S31 005047). The sites selected within these localities were at an altitude of 2,500 3,000 ft on slopes facing west to north-west, and were approximately 80 yd long by 12 yd wide, at right angles to the slope. Photographs of the three sites are shown in Figs. 1-3. Samples were taken from the foreground shown in these photographs.

METHODS

At each site a soil profile was exposed at each of 10 sampling pits, and examinations were made at, or on samples taken from, the following zones:

tussock zone (zone T)—2–4 in. deep, at the base of *Festuca* tussock plants and representing the topsoil under tussock.

inter-tussock zone (zone I)—2–4 in. deep, between well spaced *Festuca* tussock plants and representing the topsoil under inter-tussock vegetation.

subsoil zone (zone S)—12–14 in. deep, beneath zone I.

The manner of collection, and number of samples taken, for the examination of particular groups of organisms will be given in appropriate sections.

Soils were sampled, or examined, for fungi, bacteria, and protozoa, mainly in three different seasonal periods: spring (16 September–19 October 1953): summer (5 January–14 February 1954): autumn (14 April–22 May 1954). Other investigations were carried out in periods other than these and attention is drawn to this later.

Chemical analyses of the soils were performed in duplicate, by the methods described by Metson (1956) on samples collected in the summer of 1954.

Moisture determinations were made in duplicate or triplicate on samples collected at the 2–4 in. and 13–14 in. levels at the beginning

TABLE 1. Soil at Three Sites Under Low-tussock Grassland

Site	Waiouru	Bealey	Alexandra
Soil	Taupo hill soil	Tekoa steepland soil	Omarama steepland soil, moderately sloping variant
Profile description	9 in. very dark grey sandy silt; friable; weakly developed fine granular structure; many roots, very few pumice stones; boundary diffuse; 6 in. dark brown fine sandy loam; friable; weakly to moderately developed nutty structure; boundary gradual on dark yellowish brown slightly gritty fine sandy loam; friable; moderately developed fine and medium nutty structure; few pumice stones.	5 in. dark greyish brown silt loam; friable; moderately developed fine and very fine blocky and fine granular structure, boundary sharp; 10 in. brownish yellow silt loam; friable; moderately developed medium blocky and fine granular structure; occasional greywacke fragments on yellow weathered greywacke.	1 in. brownish grey fin sandy loam; very fin crumb structure masses of fine fibrou roots, boundary sharp 5 in. brownish grey fin sandy loam; mediu nutty structure; fir when dry, friabl when moist; sca tered fragments weathered schist up ½ in. diam.; mergin to 6 in. pale greyish brow fine sandy loa matrix with abunda iron stained fragmen of weathered schis 12 in. equal amounts bluish grey soft mic ceous weathered schi and of iron staine schist fragments, on bluish grey weathere schist.
Parent material	Rhyolitic volcanic ash	Greywacke	Mica schist.

and end of each sampling period and the results presented are the means of these determinations.

Temperature readings were taken in the mornings from a thermometer thrust into the profile at the 3–in. and 13–in. levels. Several readings were taken at the beginning and the end of each sampling period and the results shown are the means of these readings.

At each site the species of vascular plants occurring along the sampling transect were recorded and their frequency of occurrence assessed. A sample area, 2 ft by 18 ft, was marked off at each site and, within these areas, details concerning ground cover and number and height of tussocks were determined.

SOILS

Profile descriptions of the soils at three sites are given in Table 1, and chemical and moisture and temperature data in Table 2. The soil at the Waiouru site is formed from 9 inches or so of rhyolitic volcanic ash erupted about 1700 years ago (Ferguson and Rafter 1953), overlying mixed rhyolitic and andesitic deposits from older ash

TABLE 2. CHEMICAL ANALYSES, AND TEMPERATURE AND MOISTURE RELATIONS OF THREE SOILS UNDER LOW-TUSSOCK GRASSLAND

Site	Waiouru			Bealey			Alexandra		
Soil	Taupo hill soil			Tekoa steepland soil			Omarama steepland soil, moderately sloping variant		
Zone	T*	I	S	T	I	S	T	I	S
pH (glass electrode)	5.6	5.8	6.2	5.2	5.3	5.3	6.2	6.5	7.1
Organic carbon %	6.8	5.3	3.5	5.3	5.7	1.4	3.1	3.0	3.4
Total nitrogen %	0.55	0.48	0.32	0.33	0.34	0.11	0.27	0.28	0.31
C/N ratio	12	11	11	16	17	13	11	11	11
Citric-acid Sol. P mg. % P	10	10	7	9	7	9	17	19	6
Exchange capacity m.e.%	20.7	17.5	13.5	22.4	22.8	13.7	16.8	16.1	8.9
Total bases % m.e.%	10.3	8.1	6.0	8.7	10.4	1.5	15.8	15.9	8.5
Base saturation %	50	46	44	39	46	11	94	99	96
Exch. Ca m.e.%	8.5	6.6	4.8	6.1	7.8	1.1	11.9	11.6	5.8
Soil temperature °c									
spring	9.8		8.0	9.1		9.8	8.5		9.5
summer	16.8		15.5	18.5		15.0	20.0		16.5
autumn	7.5		9.5	9.2		11.0	12.0		12.0
Soil moisture % D.W.									
spring	58.5	48.2	50.9	58.0	55.6	37.2	22.7	21.3	14.7
summer	37.4	49.0	47.1	43.5	44.6	31.2	22.5	13.4	12.2
autumn	48.8	43.5	62.7	42.0	39.3	32.8	29.0	20.6	23.6

NOTE.—The individual cations Mg, K, and Na were also determined. The values for Taupo and Tekoa soils fell into Metson's (1956) medium-range group, while those for Omarama soil were in the medium-to-high group.

* T = tussock zone, I = inter-tussock zone, S = subsoil zone.

showers. From time to time it receives light dustings of andesitic ash from the nearby volcances but these additions are too thin to be readily observable in the profile. It is classified as a yellow-brown pumice soil (Taylor and Cox 1956) and is mapped with the hill soils of the Taupo light sandy loam (N.Z. Soil Bureau 1954, p. 82). Because of its relative youth and special kind of parent material, the soil is regarded as intrazonal, and many of its properties derive from its comparatively recent origin. Its profile, however, shows some of the characters of the zonal yellow-brown earths to which its formation is trending. Although in a suitable forest climate, the area has never been under forest, the present low tussock having been preceded by tall tussock (*Danthonia* sp.). Although very weakly weathered, the soil is weathering fairly rapidly and has a relatively high proportion of allophane in the clay fraction which may cause the high adsorption of organic matter in this soil (Fieldes 1955).

The soil at the Bealey site is formed from greywacke under beech forest (*Nothofagus cliffortioides*). It is included with the Tekoa steepland soils of the general survey of South Island. The Tekoa soils (Gibbs and Beggs 1953) belong to the weakly weathering steepland yellow-brown earths (Taylor and Cox 1956) and the soil of the site itself may be regarded as a steep phase of the weakly weathered, yellow-brown earths which are the zonal soils of the cold humid uplands. The forest on the site was removed by burning about 100 years ago, and has since been invaded by native and introduced grasses. If left ungrazed the forest would return. The soil is weakly weathered and, although strongly leached, the pH values are not as low as would be expected from the content of exchangeable bases.

The soil of the Alexandra site is formed from mica schist and is mapped with the Omarama steepland soils (Gibbs *et al.* 1954). It is a moderately sloping variant of these Omarama soils and is included with the moist brown-grey earths, which are the zonal soils of the semi-arid and near semi-arid areas. The rate of soil weathering is slow, humus content is low, and, under the low effective rainfall, leaching is weak. Soil analyses show a high content of total exchangeable bases but, for plant growth, low moisture and temperature are the limiting factors. The locality has long been under low-tussock grassland, although, since European settlement, there has been intensive grazing by sheep and rabbits and periodical burning, which has caused a serious reduction of plant cover. At the site, although the rainfall is undoubtedly higher than the 13 in. recorded at Alexandra, tussock vegetation is confined to the moist gullies and basins, bare rock and stones occurring on the more exposed ridges and slopes.

Although the soils at the two South Island sites, Bealey and Alexandra, are about equal in age, and although the vegetation at Waiouru and Alexandra has, in the past, been only tussock grassland, the soils at Waiouru and Bealey today show greater affinities than either does to the soil at the Alexandra site. This is a consequence, chiefly, of the similarity of climate, with a rainfall of over 40 in., well distributed throughout the year, and similar temperatures. It is expected that, in time, the soil at the Waiouru site would weather to a yellow-brown earth of which the soil at the Bealey site is an example.

VEGETATION

The native tussock grasslands of New Zealand (Cockayne 1928) are characterised by 'bunch' grasses, many with narrow leaves. One type, the low-tussock grasslands, is dominated by small (1–2 ft high) plants of *Festuca* and *Poa* species, and the other type, the tall-tussock grasslands, is dominated by larger (3 ft high and over) plants of *Danthonia* species. The low-tussock grasslands cover approximately 12 million acres (Cockayne and Levy 1956) and occur mainly in South Island. A general survey of these grasslands in South Island was made by Zotov (1938) and an ecological study of a particular area, Hunters Hills, South Canterbury, was made by Barker (1953). The tussocks originally formed a close cover of vegetation, but as a result of burning and grazing the tussocks are now generally well spaced, and exotic and indigenous species form the inter-tussock vegetation.

The vascular plants (club-mosses, ferns, gymnosperms, and angiosperms) occurring at the sites were recorded by A. P. Druce in the summer of 1954-55 (bryophytes and lichens were not recorded). In naming each species the most recent known revision has been used. Names in brackets () are those used in the standard local floras: Manual of the New Zealand Flora Cheeseman; A Handbook of the Naturalised Flora of New Zealand—Allan. Of the total 100 taxa (species, sub-species), only 16 occurred at all three sites, and only 27 occurred at two sites (11 at both Waiouru and Bealey, 9 at both Waiouru and Alexandra and 7 at both Bealey and Alexandra). The

TABLE 3. PHYSIOGNOMICALLY SIGNIFICANT PLANTS AT THREE SITES UNDER LOW-TUSSOCK GRASSLAND

	Waiouru	Bealey	Alexandra
Festuca novae-zelandiae	XXX	XXX	XX
Agropyron scabrum aggr. (3 taxa)	XXX	XXX	XX
Crepis capillaris	XX	XX	XXX
Danthonia nervosa (*D. pilosa* in part)	XX	XXX	—
Anthoxanthum odoratum	XXX	XXX	—
Linum cartharticum	XX	XX	—
Hypochaeris radicata	XX	XX	+
Pratia angulata	XX	—	—
Holcus lanatus	XXX	X	+
Trifolium dubium	XX	X	+
Pteridium aquilinum var. *esculentum*	—	XX	—
Agrostis tenuis	(+)	XXX	—
Poa caespitosa aggr. (2 taxa)	(+)	X	XXX
Raoulia lutescens	(+)	—	XX
Poa pratensis	—	—	XX
Cirsium arvense	+	—	XX
Aira caryophyllea	(+)	—	XX
Vulpia bromoides (*V. dertonensis*)	—	—	XX
Rumex acetosella	(+)	+	XX

Physiognomic importance XXX dominant, XX important, X less important; + unimportant, (+) rare, — absent.

greatest number, 67, occurred at only a single location (27 at Waiouru, 16 at Bealey and 24 at Alexandra). Table 3 lists only the physiognomically important species so that a clearer picture of the significant differences in floristic composition may be obtained. Table 4 indicates the size and cover of the tussocks and inter-tussock vegetation, and records other features at the sites.

TABLE 4. VEGETATIONAL AND OTHER FEATURES OF THREE LOCALITIES UNDER LOW-TUSSOCK GRASSLAND

Locality	Waiouru	Bealey	Alexandra
*Total cover	Near 100%	c. 90%	c. 50%
*Basal cover of tussock (at ground level)	3%	4%	6%
*Number of tussocks	30 (30 *Festuca*)	43 (30 *Festuca*/13 *Poa*)	i 27 (12 *Festuca*-15 *Poa*) ii 27 (4 *Festuca*-23 *Poa*)
*Height of tussocks (excl. culms)	Mean 12 in., max. 16 in.	Mean 10 in., max. 8 in.	Mean 10 in., max. 21 in.
*Basal diam. tussocks	Mean 2.3 in., max. 6 in.	Mean 1.7 in., Max. 8 in.	Mean 3.6 in., max. 10 in.
*Inter-tussock vegetation	Tall grass (culms ± 24 in.) with flatweeds and other herbs (many creeping) forming a dense cover 0–10 in. high.	Tall grass (culms ± 14 in.) and short *Pteridium*, with light ground cover of flatweeds and other herbs ± 6 in. high.	Very sparse layer of flatweed heads, *Aira* and *Vulpia* ± 6in. 50% ground cover of *Raoulia*, flatweeds, *Rumex* and other herbs: *Cirsium arvense* and *Poa pratensis* locally conspicuous.
Total number of species	63 (48 indig/15 exotic)	50 (38 indig./12 exotic)	56 (38 indig./18 exotic)
Number of woody species	3 (5%)	8 (16%) (Wood fragments in soil)	2 (4%)
Altitude	c. 3,000 ft.	c. 2,400 ft.	c. 3,000 ft.
Slope	c. 25°	c. 33°	10–15°
Aspect	W–WNW	NW	NNW–N
Rainfall (avge. annual)	48″ (recorded at Waiouru)	65″ (recorded at Cass)	13″ (recorded at Alexandra).
Burning	Burnt in past	Burnt in past	Burnt in past.
Grazing	Very light (sheep)	Moderate (sheep)	Severe (rabbits).
Trampling	Few sheep tracks	Numerous sheep tracks	Negligible.
General description of present vegetation	Grassland with scattered *Festuca* tussocks, most of them young.	Grassland with scattered *Festuca* tussocks, most of them young.	Grassland with scattered tussocks, mainly *Poa*, most of them old.
Surrounding vegetation types other than that on locations	*Danthonia-Festuca* modified tussock grassland.	*Nothofagus cliffortioides* forest, and manuka scrub with young beech.	Scabweed and bare ground.
Original vegetation	Subclimax *Danthonia* tall-tussock grassland with a small amount of *Festuca*	Climax *Nothofagus cliffortioides* forest.	Climax *Festuca-Poa* low-tussock grassland.

* In sample area 18 ft by 2 ft.

The life-form composition of the vegetation at the three sites was essentially similar, the main differences being a greater percentage of phanerophytes at Bealey and a greater percentage of annuals at Alexandra. The structure of the three communities was very similar, differences being related to differences in the grazing pressure. The dynamics of the tussock populations differ, however, young tussocks being present at Waiouru and Bealey but not at Alexandra. Under the present conditions the tussock vegetation at the latter site is in a state of senility. In summary it may be said that the vegetation at the three sites is similar structurally, though different floristically. Waiouru and Bealey sites have a greater number of physiognomically important species in common than either has with the Alexandra site.

Originally, tussock grasslands carried no clovers and few other legumes. At present, clovers are invading these areas and their establishment is being encouraged by graziers. For this reason and others mentioned above, it is apparent that these areas of tussock grassland are much modified remnants of the original cover.

From the above examination of the soils and vegetation at the three sites it can be seen that the site near Alexandra differed quite strongly from those at Waiouru and Bealey, which showed similarities to each other.

ACKNOWLEDGEMENTS

The author is indebted to the following colleagues in the Soil Bureau for assistance: Messrs. M. L. Leamy, J. P. Fox, and J. D. McCraw for soil descriptions: Miss K. M. Slocombe for soil analyses; Mr. A. P. Druce for the examination and analysis of the vegetation, and provision of Tables 3 and 4. Thanks are due to Mr. N. H. Taylor for encouragement and helpful criticism throughout this series of investigations.

REFERENCES

BARKER, A. P. 1953: An Ecological Study of Tussock Grassland, Hunters Hills, South Canterbury. *N.Z. Dep. sci. industr. Res. Bull. 107.* 58 pp.

COCKAYNE, A. H.; LEVY, E. B. 1956: Grasslands of New Zealand. *Proc. 7th int. Grassl. Congr.* pp. 585-611.

COCKAYNE, L. 1928: The Vegetation of New Zealand in "Die Vegetation der Erde." Vol. 14. Leipzig.

FERGUSON, G.; RAFTER, T. A. 1953: New Zealand ^{14}C Age Measurements. *N.Z. J. Sci. Tech. 35:* 127-8.

FIELDES, M. 1955: Clay Mineralogy of New Zealand Soils. II. Allophane and Related Mineral Colloids. *N.Z. J. Sci. Tech. 37:* 336-50.

GIBBS, H. S.; BEGGS, J. P. 1953: Soils and Agriculture of Awatere, Kaikoura, and Part of Marlborough Counties. *N.Z. Soil Bur. Bull. 9.*

GIBBS, H. S.; RAESIDE, J. D.; DIXON, J. K.; METSON, A. J. 1945: Soil Erosion in the High Country of the South Island. *N.Z. Dep. sci. industr. Res. Bull. 92.*

METSON, A. J. 1956: Methods of Chemical Analysis for Soil Survey Samples. *N.Z. Soil Bur. Bull. 12.*

N.Z. SOIL BUREAU 1954: General Survey of the Soils of North Island, New Zealand. *N.Z. Soil Bur. Bull. n.s. 5.* 286 pp.

TAYLOR, N. H.; COX, P. E. 1956: The Soil Pattern of New Zealand. *Proc. N.Z. Inst. agric. Sci.*

ZOTOV, V. D. 1938: Survey of the Tussock Grasslands of the South Island, New Zealand. *N.Z. J. Sci. Tech. A20:* 212-44.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

II. FUNGI

By R. H. THORNTON, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 19 June 1958*)

Summary

The fungi occurring in three soils, under low-tussock grassland, were examined at various seasons, employing screened immersion plate, soil plate, and rhizosphere and root plating, techniques. Approximately 100 species were isolated.

The screened immersion plate method showed that: (a) the three soils were of similar mycelial status; (b) maximum growth was obtained in summer; (c) non-sporing fungi, including *Rhizoctonia* sp. and *Papulaspora* sp., accounted for approximately 60% of all isolations from the three soils; (d) *Rhizoctonia* sp. dominated all three soils.

Soil fungal patterns, based on percentage frequency of occurrence, are shown for the three soils.

The broad relationships of the soil fungal floras reflect, generally, the broad relationships between the vegetations and between the soils.

Fungal isolations by the soil plate method differed from screened plate isolations, particularly in the absence of *Rhizoctonia* sp.

Rhizosphere platings from tussock plants gave similar results to soil plate isolations.

Platings of tussock plant roots were dominated by *Trichoderma viride* and by members of the Mucorales.

It is suggested that the soil fungal populations are greatly influenced by the higher-plant roots.

INTRODUCTION

An investigation of the fungi occurring in three tussock-grassland soils was carried out as part of a series of microbiological investigations of these soils (Thornton 1958). Several methods were employed: screened immersion plates (Thornton 1952), soil plates (Warcup 1950), and rhizosphere and root platings. Results by these methods will be considered separately. A list of the species isolated is given in the Appendix.

FUNGI BY SCREENED IMMERSION PLATES

The method employed was essentially that described earlier with several modifications (Thornton 1956a). The isolating medium was 2% distilled-water agar, and mycelial growth on agar films was transferred to slopes of acidified potato dextrose agar for subsequent identification.

One plate was employed in each of the three soil zones, in each of ten sample pits at three sites in the spring, summer, and autumn

N.Z. J. agric. Res. 1: 922–938.

seasons of 1953-54 (Thornton 1958). A total of 270 screened plates, 10 apertures per plate, was used. Plates were buried for 7 days in spring and autumn and 4 days in summer.

Mycelia in Soils

The distribution of mycelia in the three soils is given in Table 1.

TABLE 1. NUMBERS OF MYCELIA ISOLATED BY SCREENED IMMERSION PLATES FROM THREE ZONES IN THREE TUSSOCK-GRASSLAND SOILS IN THREE SEASONS

Soil	Zones	Spring	Summer	Autumn	Total
Taupo soil (Waiouru)	Tussock	63	81	84	228
	Inter-tussock	78	86	72	236
	Subsoil	26	48	48	122
		167	215	204	586
Tekoa soil (Bealey)	Tussock	64	82	58	204
	Inter-tussock	58	82	51	191
	Subsoil	31	54	41	126
		153	218	150	521
Omarama soil (Alexandra)	Tussock	42	97	53	192
	Inter-tussock	72	82	47	201
	Subsoil	59	60	50	169
		173	239	150	562
					1669

If the number of mycelia isolated is regarded as a measure of the relative mycelial status of the soils, then the three soils appear to be similar, for of a total of 1669 mycelia isolated from the three soils, 586 were from the Taupo soil, at Waiouru, 521 from the Tekoa soil at Bealey, and 562 from the Omarama soil near Alexandra. This similarity is not due to the colonising of all apertures in the screened plates by mycelia, because at each site 90 plates, each with 10 apertures, i.e. 900 apertures, were employed, yet less than 600 mycelia were isolated.

Season appeared to affect the numbers of isolations in that in all three soils the greatest number of isolations were recorded in summer. This is in agreement with previous work on oakwood and heath soils (Thornton 1956b).

The different burial periods employed in the various seasons to obtain suitable growth for isolation tend to confirm this favourable effect of summer. In general, the numbers of mycelia isolated showed a correlation with temperature. Eggleton (1938) suggested that such seasonal effects were indirect, and reflected changes in the growth of the higher-plant cover which affected the amount of energy material available to micro-organisms.

The total numbers of mycelia isolated from tussock and inter-tussock zones in the three soils were similar, i.e., about 200–230, while in the subsoil zone counts were consistently lower, 120–170. The differences in counts between topsoil and subsoil zones varied from soil to soil and from season to season. In general, the Omarama soil showed least difference and the Taupo soil the greatest.

Distribution of Species

The number of identified species in the three soils was similar, i.e., Taupo 46, Tekoa 48, Omarama 53. Many specimens could not be identified. Although they grew well, many producing white mycelia and others dark, none formed spores after many months in culture on a variety of media. Several produced sclerotia but none produced clamp connections.

The distribution in the three soils of those fungi which contributed more than 1% of total isolations from any one soil, is given in Table 2.

TABLE 2. TOTAL NUMBERS OF MYCELIA OF FUNGI ISOLATED FROM TUSSOCK-GRASSLAND SOILS BY SCREENED IMMERSION PLATES IN THREE SEASONS, WHICH CONTRIBUTE 1% OR MORE OF ISOLATIONS FROM ANY ONE SOIL

	Tekoa Soil (Waiouru)	Tekoa Soil (Bealey)	Omarama Soil (Alexandra)
Rhizoctonia sp.	243	193	312
Cylindrocarpon radicicola	118	36	4
Other non-sporing mycelia	27	37	49
Fungus S.B. 235	33	56	—
Papulaspora sp.	18	24	1
Trichoderma viride	7	23	6
Penicillium janthinellum	4	12	15
Fusarium sambucinum var. *coeruleum*	7	—	21
Absidia glauca	—	2	22
Zygorhynchus moelleri	7	16	—
Penicillium stoloniferum	5	11	7
Fusarium oxysporum	16	7	—
Mortierella alpina	7	6	7
Fusarium avenaceum	—	7	11
Mortierella elongata	7	2	9
Mortierella minutissima	11	4	2
Cladosporium herbarum	2	9	6
Verticillium spp.	6	5	6
Penicillium cyclopium	4	6	5
?*Pythium* spp.	6	4	5
Cylindrocarpon olidum	5	7	2
Trichocladium opacum	2	6	2
Candida humicola	6	2	—
Pestalotia sp.	—	1	7
Penicillium nigricans	1	5	1
Mortierella horticola	7	—	—

If each species was given a constancy rating, i.e., a fungus was given a rating of (1) for every screened plate it was isolated from, irrespective of the number of isolations of that species from that plate, a strong correlation between frequency of occurrence and constancy rating appeared for all species. Therefore, constancy ratings have been omitted in this account. Of the 24 species in Table 2, 17 were common to all three soils, 6 were common to any two soils, and one (*Mortierella horticola*) was isolated from Taupo soil only. This relatively small list of species accounted for 73% of the mycelia isolated from all three soils.

The outstanding feature of the results was the dominance by *Rhizoctonia* mycelia: of a total of 1669 mycelia, 748 or approximately 45%, were *Rhizoctonia*. (Fungus S.B. 235 was provisionally placed under *Rhizoctonia* sp. in an earlier communication (Thornton 1956a)). This is at variance with the relatively infrequent occurrence of *Rhizoctonia* in other records of soil fungal isolations. The presence of *Rhizoctonia* mycelia in these soils was confirmed by the use of Rossi-Cholodny slides and by agar soil films devised by Jones and Mollison (1948). In addition, blocks of soil were brought to the laboratory and broken open along natural fracture lines. Microscopical examination of freshly exposed surfaces revealed *Rhizoctonia* mycelia (Thornton 1956a).

When the non-sporing fungi, *Rhizoctonia, Papulaspora,* and fungus S.B. 235, are grouped with the unidentified 'other non-sporing mycelia' they form approximately 60% of all isolations from the three soils. Warcup (1957), also, found that 70% of fungi obtained by a hyphal isolation method from a wheat-field soil in Australia were non-sporing forms, including *Rhizoctonia*. *Rhizoctonia* mycelium was isolated also by Chesters (1948). It would appear that non-sporing fungi are more important in soils than has hitherto been realised.

Table 3 shows the distribution of isolations of the more abundant species according to soil type, zone, and season.

Specific Nature of the Population

The specific nature of the fungal population will be discussed in the following under the headings of the broad taxonomic groups of fungi.

PHYCOMYCETES: Members of this group were frequently isolated and contributed a little over 8% of the total isolations. Species of *Mortierella* were dominant and it would appear that members of this genus are typical components of soil fungal populations (Bisby *et al.* 1935; Warcup 1951; McLennan and Ducker 1954; Thornton 1956b; Warcup 1957). *Mortierella alpina* in particular appears to be of widespread occurrence. Although *Absidia glauca* was absent from the Taupo soil and *Zygorhynchus moelleri* was absent from the Omarama soil, both were frequently isolated from the other two soils. *A. glauca* was isolated with higher frequency from the tussock zones of Omarama and Tekoa soils, which may indicate that this fungus was selectively stimulated by the roots of the tussock plants. *Z. moelleri* appeared to be more common in summer than in any other season.

TABLE 3. OCCURRENCE OF FUNGI FREQUENTLY ISOLATED BY SCREENED IMMERSION PLATES FROM THREE ZONES OF THREE TUSSOCK-GRASSLAND SOILS AT THREE SEASONS
(Total numbers of mycelia isolated)

		Taupo Soil (Waiouru)			Tekoa Soil (Bealey)			Omarama Soil (Alexandra)		
	Season	*Sp	S	A	Sp	S	A	Sp	S	A
	Zones:									
Rhizoctonia sp.	T	35	8	46	18	12	21	19	33	38
	I	34	23	36	19	24	29	46	40	32
	S	16	22	25	29	19	22	39	28	37
Cylindrocarpon radicicola	T	8	16	21	11	1	5	—	—	
	I	13	24	12	9	—	1	—	3	—
	S	4	8	12	—	2	7	1	—	—
Other non-sporing mycelia	T	4	8	—	9	9	3	6	8	1
	I	5	1	3	8	3	1	2	8	4
	S	4	1	1	—	3	1	8	9	3
Fungus S.B. 235	T	3	—	3	—	10	4	—	—	—
	I	8	1	10	—	16	5	—	—	—
	S	—	3	5	—	12	9	—	—	—
Papulaspora sp.	T	1	1	1	—	6	—	—	—	—
	I	2	2	4	5	12	1	—	1	—
	S	1	3	3	—	—		—	—	—
Trichoderma viride	T	1	6	—	2	10	3	—	1	1
	I	—	—	—	—	6	2	1	—	1
	S	—	—	—	—	—	—	—	2	—
Penicillium janthinellum	T	—	1	—	—	5	3	2	1	3
	I	1	—	—	1	1	2	—	5	1
	S	—	2	—	—	—	—	2	1	—
Penicillium stoloniferum	T	—	1	—	5	—	—	4	—	—
	I	1	2	—	5	—	—	—	1	—
	S	—	1	—	1	—	—	—	1	1
Penicillium cyclopium	T	1	1	—	6	—	—	2	—	—
	I	1	—	—	—	—	—	3	—	—
	S	—	1	—	—	—		—	—	—
Fusarium sambucinum var. *coeruleum*	T	—	1	—	—	—	—	1	5	—
	I	2	3	1	—	—	—	7	3	—
	S	—	—	—	—	—	—	4	1	—
Fusarium oxysporum	T	—	3	1	1	1	—	—	—	—
	I	4	7	1	3	2	—	—	—	—
	S	—	—	—	—	—	—	—	—	—
Fusarium avenaceum	T	—	—	—	—	4	—	—	6	1
	I	—	—	—	—	2	1	—	4	—
	S	—						—	—	—
Absidia glauca	T	—	—	—	—	1	1	—	13	—
	I	—	—	—	—	—	—	3	3	1
	S	—	—	—	—	—	—	—	1	1
Zygorhynchus moelleri	T	—	2	—	1	6	2	—	—	—
	I	—	5	—	1	1	—		—	—
	S	—	—	—	—	5			—	—
Mortierella alpina	T	1	1	1	2	1	—		—	—
	I	—	3	—	1	1	—	—	3	3
	S	—	—	1	—	1	—	1	3	—
Mortierella elongata	T	2	2	—	—	—	—	1	2	1
	I	1	2	—	—	—	1	—	2	3
	S	—	—	—	—	1	—	—	—	—
Mortierella minutissima	T	1	6	—	—	—	2	—	—	—
	I	—	4	—	—	—	—	—	1	1
	S	—	—	—	—	—	2	—	—	—
Mortierella horticola	T	2	4	—	—	—	—	—	—	—
	I	—	—	—	—	—	—	—	—	—
	S	—	—	—	—	—	—		—	

* Sp = Spring, S = Summer, A = Autumn.

ASCOMYCETES: Members of this group were not frequently isolated from these soils. A number of yeasts were isolated from screened plates, and although two species form mycelium and therefore could grow through apertures, viz., *Candida humicola* and *Trichosporon cutaneum*, the others are probably carried through apertures on hyphae of other fungi. Bacteria are often carried on to plates in this manner and this has necessitated the use of an acid enriched agar when first transferring growth from screened plates.

FUNGI IMPERFECTI: In common with a majority of soil fungal investigations, members of this group were very frequently isolated. *Trichoderma viride* was present in the three soils, commonly in Tekoa soil where highest numbers occurred in the tussock zone, mainly in summer. The genus *Penicillium* contributed approximately $7\frac{1}{2}\%$ of total isolations, in which three species, *P. janthinellum, P. stoloniferum*, and *P. cyclopium* were dominant. These three fungi occurred more frequently in the two South Island soils where, in general, year-round soil temperatures are lower. Three species of *Fusarium* were isolated and showed a distinct distribution pattern; each species was absent from a different soil and present in the other two. No explanation can be offered for this marked distribution. Employing the same isolation technique, *Fusarium* spp. were previously found to be absent from oakwood and heath soil profiles (Thornton 1956b). Two species of *Cylindrocarpon* were isolated; *C. radicicola* was very frequently recovered from the Taupo soil, less frequently from Tekoa soil and still less frequently from Omarama soil, while *C. olidum* occurred less frequently in all three soils.

MYCELIA STERILIA: The occurrence of *Rhizoctonia* mycelia has already been commented upon. Two species were recognised by Dr. A. Kerr, Waite Institute, Adelaide, South Australia, to whom cultures were sent. An unnamed *Rhizoctonia* sp. was present in all three soils and was by far the most common fungus in these soils. A strain of *Rhizoctonia solani* was isolated from Omarama soil only, but records

TABLE 4. OCCURRENCE OF MYCELIA OF *Rhizoctonia* SP. IN ZONES OF THREE TUSSOCK-GRASSLAND SOILS

	Taupo Soil (Waiouru)			Tekoa Soil (Bealey)			Omarama Soil (Alexandra)		
Zones:	T	I	S	T	I	S	T	I	S
Total number of *Rhizoctonia* mycelia isolated	87	93	63	51	72	70	90	118	104
Rhizoctonia mycelia expressed as a percentage of the total number of mycelia isolated	39	39	52	28	38	56	47	58	62

do not show clearly the distribution of the very small number of isolations and they have therefore been grouped with the unnamed *Rhizoctonia* sp. Whereas most species decreased in number in the subsoil zone, *Rhizoctonia* mycelia showed a more even distribution throughout the depth of the profile. Table 4 gives the total and percentage occurrence of *Rhizoctonia* mycelia in the various zones of the three soils. In the Tekoa and Omarama soils, there was a slight proportional increase of *Rhizoctonia* mycelia in the inter-tussock zone compared with the tussock zone and in all three soils there was a marked relative percentage increase in the subsoil zone.

Fungus S.B. 235 has been shown separately from the 'other non-sporing' fungi because of its high frequency and its characteristic growth. Small sclerotia, off-white in colour, usually 1–3 mm in diameter were formed in and upon the medium and upon the aerial, white, cottony mycelium which was composed of septate, even, hyphae, 2–3 μ in diameter. The fungus was absent from the Omarama soil but present in the other two, higher numbers being recorded in the Tekoa soil. There, summer was the most favourable season, although in Taupo soil highest numbers were recorded in the autumn.

A species of *Papulaspora* was isolated quite often, mainly from inter-tussock zones of Taupo and Tekoa soils. No known specific descriptions fit it and no antibiotic activity toward the *Rhizoctonia* sp. isolated has been detected (Warren 1948).

General Nature of the Population

In these perennial-grassland soils, which abound with roots and in which the main organic-matter additions are of root origin, the fungal population must be considerably influenced by grass roots and may possibly be regarded as being rhizospheric. (This is discussed later.) The lack of important differences between tussock and inter-tussock zone mycofloras, both in numbers of mycelia isolated and the species present, suggests that there is little difference, in the influence on fungi, between *Festuca* tussock and the inter-tussock vegetation, which is mainly other grasses.

Soil Fungal Patterns

A concept of soil fungal patterns, based on percentage frequency of occurrence of species, has been proposed for studies on soil fungal ecology, and patterns including those species contributing more than 5% of total isolations from a heath and a forest soil have been prepared (Thornton 1956b). The limit of 5% was arbitrarily chosen for convenience in demonstrating the pattern approach. In the present investigation, patterns have been prepared of those species contributing more than 2% of isolations (Table 5). If the level of 5% frequency of occurrence had been employed in the present study, three fungi only would have been recorded in the patterns: *Rhizoctonia* sp., dominant in all three soils, Fungus S.B. 235, and *Cylindrocarpon radicicola*, subdominant in Taupo and Tekoa soils; at a 2% level, other species enter the pattern. The species shown in the patterns, including the 'other non-sporing' forms, contribute more than 75% of the total

TABLE 5. SPECIES CONTRIBUTING MORE THAN 2% OF ISOLATIONS FROM SCREENED IMMERSION PLATES, FOR THREE TUSSOCK-GRASSLAND SOILS
(Numbers are percentage of total isolations)

Taupo Soil (Waiouru)		Tekoa Soil (Bealey)		Omarama Soil (Alexandra)	
Rhizoctonia sp.	41.5	*Rhizoctonia* sp.	36.8	*Rhizoctonia* sp.	55.6
Cylindrocarpon radicicola	20.1	Fungus S.B. 235	10.7	Other non-sporing mycelia	8.7
Fungus S.B. 235	5.6	Other non-sporing mycelia	7.1	*Absidia glauca*	3.9
Other non-sporing mycelia	4.4	*Cylindrocarpon radicicola*	6.9	*Fusarium sambucinum* var. *coeruleum*	3.7
Papulaspora sp.	3.1	*Papulaspora* sp.	4.6	*Penicillium janthinellum*	2.7
Fusarium oxysporum	2.7	*Trichoderma viride*	4.4		
		Zygorhynchus moelleri	3.8		
		Penicillium janthinellum	2.3		
		Penicillium stoloniferum	2.1		

isolations from each soil. A characteristic feature of the patterns is the relatively small number of species which contribute the great bulk of mycelia. This lends support to an earlier suggestion that in relatively undisturbed soils a small number of species assume dominance as a result of particular, favourable conditions (Thornton 1956b).

The three soil fungal patterns appear to be basically similar as each is dominated by one or two species only and, in each, the list of species is very small. When the species forming the pattern are considered, the similarity of the patterns is shown further.

All three soils are dominated by *Rhizoctonia* sp. and the majority of the 24 common species are present in all three soils, the remainder being present in at least two soils. The increased incidence of *A. glauca* in the Omarama scil may be due to selective stimulation in the tussock zone, and the greater frequency of *T. viride* and *Z. moelleri* in the Tekoa soil may be correlated with the leached, more acid soil, or may reflect the past history of forest vegetation on this site. The generic patterns of the common species in the three soils are indistinguishable. It is concluded that the fungal patterns of the three soils show close similarities, those for the Taupo and Tekoa soils showing even closer affinities because both are dominated by the same four species and the less-common fungi occur in similar proportions in both soils. A strong relationship between the soil fungal flora and the higher-plant cover was found also by Orpurt and Curtis (1957) working with prairie grasslands in America.

The fungal patterns of the three tussock-grassland soils differ quite strongly from those described elsewhere (Thornton 1956b). That for an oakwood soil was dominated by *Trichoderma viride* and one for a heath soil was dominated by *T. viride* and *Mucor ramannianus*. This may reflect an influence by higher-plant cover on soil fungal populations.

Ecological Factors

The main ecological factors likely to influence the composition of the fungal population are type and supply of nutrients, moisture, and temperature. The range of soil moistures and temperatures encountered in these soils appeared to affect, principally, number of mycelia, and possibly had only slight qualitative effects. It is concluded that type and supply of nutrients are the principal factors and these will be controlled, largely, by the higher plants, through the roots, both living and dead. The similar mycelial status of the three soils (Table 1) suggests that the supply of nutrients is similar in each, so that probably type of nutrients accounts for the differences recorded. At Waiouru and Bealey, where the percentage plant cover and the composition of the cover are similar, the fungal patterns are similar. At Alexandra, however, where the plant cover is less and there is a greater proportion of annuals, the fungal pattern is a little different from these two. It is not known to what extent fungal populations are influenced by the chemical composition of the soil, but such effects are probably indirect, being reflected through changes in the growth and composition of the plant cover. It is interesting to find that the broad similarities of the soil fungal floras reflect, generally, the broad relationships between the vegetations and between the soils of the three sites (Thornton 1958).

Whereas the taxonomy of fungi is based largely on morphology, some classifications of other organisms, yeasts (Lodder and van Rij 1952), and bacteria (Hugh and Leifson 1953), employ physiology as an aid. For these, patterns possibly having physiological significance for soil biology may be prepared. For fungi, which are able to grow on a wide range of substrates and are very adaptable (Foster 1949), taxonomically similar patterns may not necessarily represent similar physiological patterns because little work has been done to link physiology with taxonomy. Thus, no helpful generalisation of the activities of the fungi in these soils can be made from these results apart from stating that they decompose plant and animal remains. For this same reason similar taxonomic patterns may not necessarily be safe criteria for judging the habitats to be the same. This also applies to higher plants, e.g. grasses (McMillan 1956). In an attempt to gain information on the activity of the common fungi in these soils, nutritional and physiological studies are being carried out in these laboratories and results will be presented at a later date.

FUNGI BY SOIL PLATES

A plating method of isolating fungi, described by Warcup (1950), was employed in parallel studies with investigations by screened immersion plates. Composite samples were obtained from each of three zones, in three soils, in spring and autumn seasons of 1953-54, at the same time as screened plates were buried. Five sites were sampled in each soil type and three plates were inoculated from each sample. A total of 270 plates were examined and representative fungi were cultured on slopes.

The numbers of species recorded from each soil were very similar although the constancy of occurrence and their relative incidence in the three soils varied. Table 6 shows the occurrence of frequently isolated fungi in the three soils. A fungus was considered frequent if it occurred in six or more samples. This figure was arbitrarily chosen and has no other significance. (Other fungi isolated are given in the Appendix.) Fungi which, to date, have not produced any spores in culture were labelled 'other non-sporing mycelia' (i.e. other than *Papulaspora* sp., *Rhizoctonia* sp., and fungus S.B. 235). They were often isolated and many formed white mycelium and several were dark coloured. Of the 64 species isolated, 13 were common to all three soils, 25 were isolated from at least two soils, and 26 came from only one of the three soils.

TABLE 6. OCCURRENCE OF FUNGI FREQUENTLY ISOLATED FROM SOIL PLATES, IN THIRTY SAMPLES FROM EACH OF THREE TUSSOCK-GRASSLAND SOILS, IN ORDER OF FREQUENCY

	Taupo Soil (Waiouru)	Tekoa Soil (Bealey)	Omarama Soil (Alexandra)
Other non-sporing mycelia*	20	14	21
Absidia glauca	6	14	22
Trichoderma viride	11	18	12
Penicillium nigricans	13	10	15
Zygorhynchus moelleri	7	22	2
Penicillium janthinellum	9	10	8
Mortierella minutissima	5	10	1
Penicillium cyclopium	1	10	3
Pestalotia sp.	4	2	5
Mucor hiemalis	—	3	7
Mortierella alpina	7	1	2
? *Pythium* spp.	7	1	2
Humicola grisea	7	—	3
Trichocladium opacum	8	2	—
Penicillium frequentans	3	5	—
Penicillium corylophilum	1	2	4
Penicillium chrysogenum	—	6	1
Mortierella horticola	6	—	—
Papularia sphaeronema	1	3	2
Arachniotus sp.	4	—	2
Mortierella elongata	2	—	4

* Other than *Papulaspora* sp.; *Rhizoctonia* sp. or fungus S.B. 235.

The following fungi were commonly isolated from all three soils: *Absidia glauca, Trichoderma viride, Penicillum nigricans, Zygorhynchus moelleri, Penicillium janthinellum*, and *Mortierella alpina*. The higher relative occurrence of *A. glauca* in the Omarama soil, and of *Z. moelleri* in the Tekoa soil was quite marked. *T. viride* appeared to be a little more common in the Tekoa soil. The relative distribution of these fungi was similar to that shown by results from screened immersion plates. *P. cyclopium* and *Mortierella minutissima* were more frequently isolated from Tekoa soil. *Mortierella horticola* was isolated only from Taupo soil by both soil plate and screened plate techniques.

There is little to indicate, in the number of species and their occurrence, that season influenced the fungal population, although the numbers of isolations of representative colonies from culture plates showed an increase in autumn. Generally, in the subsoil zone fungi were less frequent, although *Z. moelleri, M. minutissima, P. cyclopium,* and *Trichocladium opacum* were isolated with equal frequency from both upper and lower zones. *A. glauca* occurred with greater frequency in the tussock zone samples and, apart from this, there was little difference between tussock and inter-tussock zone fungal populations.

In grassland soils dominated by *Festuca* and *Agrostis* species Warcup (1951) found a larger number of species than has been recorded in this investigation. The following fungi, common to the present study, were isolated frequently also by Warcup: *Absidia glauca, Mortierella alpina, M. minutissima, Penicillium nigricans,* and *Trichoderma viride.*

In comparing these results with those gained by screened immersion plates, the absence of some fungi, particularly *Rhizoctonia* sp. and S.B. 235, and the marked change in incidence of others, indicate that the two methods reflect different aspects of the soil fungal floras. It is generally considered that plating methods favour heavily sporing fungi (Garrett 1951). Warcup (1955) developed a method for determining the nature of the propagules (i.e., spore or hyphae) that give rise to individual colonies of fungi on soil dilution plates and found that the majority of colonies developed from spores. It is possible that the soil plate technique suffers the same disadvantage. Recent studies by Warcup (1957) indicate that this is so.

The present investigation suggests that the soil plate method is unable to give a true indication of the active fungal flora of these soils (Chesters and Thornton 1956).

FUNGI BY RHIZOSPHERE AND ROOT PLATINGS

At each sampling location a number of tussock plants were dug up and most of the bulk of soil adhering to roots was shaken off. All roots and adhering soil were cut off near the crown of the plants and placed in sterile containers. Two treatments were then carried out: (a) from the root mass of each plant five healthy roots were selected and a 3–5 cm portion cut off from each and placed separately in a sterile 250 ml flask; 100 ml sterile tap water was added to each flask and root portions washed by vigorous hand shaking for three minutes. The liquid was decanted and the washing procedure repeated for five successive washings. Root portions were then placed in sterile petri dishes and overpoured with sterile melted and cooled Czapek-Dox solution agar plus yeast extract, adjusted to pH 4. After incubation at room temperature, representative colonies were subcultured on slopes for subsequent identification. This procedure constituted "root platings". (b) To the container with the remaining roots and adhering soil, 250 ml sterile tap water was added and the suspension shaken vigorously by hand for seven minutes. This gave an approximate initial dilution of 1/50, calculated from the dry weights of roots and soil. After serial dilution to 1/5,000, 1 ml portions were added to sterile petri dishes and overpoured with Czapek-Dox solution agar plus yeast extract (pH 4), and incubated at room temperature. From each plant two dilution series were prepared and five petri dishes were inoculated for each series. This constituted "rhizosphere platings".

Two plants were dug up in each of three sites in both spring and summer seasons. A total of 12 plants were used, 60 root portions were examined and 120 plates were inoculated. Representative colonies were subcultured on slopes.

Rhizosphere Platings

Rhizosphere platings were made from plants of *Festuca novae-zelandiae*. The relative occurrence of the various fungi was assessed by a rating method. A fungus was given a rating of (1) if it occurred on one or more of 10 petri dishes prepared from the dilutions of root and adhering soil of one plant. Thus, at any location the maximum rating for any fungus was 4. Table 7 shows the more frequently isolated fungi from rhizosphere platings of plants collected at the three sites. (Other fungi isolated are given in the Appendix.) Whereas the

TABLE 7. OCCURRENCE RATING OF FUNGI FREQUENTLY ISOLATED IN RHIZOSPHERE PLANTINGS FROM PLANTS OF *Festuca novae-zelandiae* ON THREE SOILS, IN ORDER OF OCCURRENCE

	Taupo Soil (Waiouru)	Tekoa Soil (Bealey)	Omarama Soil (Alexandra)
Trichoderma viride	4	4	3
Penicillium nigricans	4	4	3
Other non-sporing mycelia*	3	4	4
Absidia glauca	2	4	4
Penicillium janthinellum	3	1	4
Mortierella alpina	3	2	2
Penicillium novae-zelandiae	—	4	3
Fusarium avenaceum	—	2	4
Papularia sphaeronema	2	2	2
Mortierella minutissima	3	3	—
Penicillium citrinum	4	1	—
Penicillium thomii	3	—	2
Cladosporium sp.	3	—	2
Zygorhynchus moelleri	2	2	1
Fusarium sambucinum var. *coeruleum*	3	—	2

* Other than *Papulaspora* sp.; *Rhizoctonia* sp. or fungus S.B. 235.

results by screened immersion plates showed *Rhizoctonia* sp. in the root zone, this fungus did not appear in rhizosphere platings. This may have resulted from a selective effect by the medium employed, which could also account for the fact that the fungi common in rhizosphere platings were also commonly isolated from inter-tussock zone samples by the soil plate technique. The similarity of these results also suggests an apparent lack of a distinct rhizosphere flora, although it is felt that the soil fungal population must be considerably influenced by the higher-plant roots. This is discussed later.

Root Platings

Fungi on the root surface of plants of *F. novae-zelandiae* and of *Poa caespitosa* were examined by the method already described. Two plants only of *P. caespitosa*, collected in the autumn, were examined.

TABLE 8. OCCURRENCE RATING OF FUNGI FREQUENTLY ISOLATED FROM ROOTS OF *Festuca novae-zelandiae* AND OF *Poa caespitosa* ON THREE SOILS, IN ORDER OF OCCURRENCE

	Taupo Soil (Waiouru)		Tekoa Soil (Bealey)		Omarama Soil (Alexandra)	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Festuca novae-zelandiae						
Other non-sporing mycelia*	6	5	1	5	3	2
Absidia glauca	2	2	10	4	8	1
Mucor hiemalis	—	2	5	3	3	10
Zygorhynchus moelleri	—	5	2	8	3	—
Trichoderma viride	4	3	3	1		
Mortierella minutissima	3	1	—	3		2
Poa caespitosa						
Other non-sporing mycelia*		6		8		4
Trichoderma viride		8		2		—
Mucor hiemalis				4		4
Mucor nr *sphaerosporus*		—		3		5
Absidia glauca		-		—		6
Zygorhynchus moelleri		—		3		1
Penicillium janthinellum		—		1		3

* Other than *Papulaspora* sp.; *Rhizoctonia* sp or fungus S.B. 235.

The relative occurrence of fungi on roots was assessed by giving a rating of (1) to a fungus if it was isolated from a root length. Five root lengths were taken from each plant collected, and two plants were collected in each of two seasons.

Table 8 shows the more frequently isolated fungi from the root surface of tussock plants collected at three sites. (Other fungi isolated are given in the Appendix.) Members of the Mucorales and *Trichoderma viride* were the most common fungi on root portions of both *F. novae-zelandiae* and *P. caespitosa.* Other fungi were present but occurred on less than half the number of plants examined. It is known from the results by screened immersion plates that hyphae of *Rhizoctonia* were present in these soils in the tussock zones yet the root-plating procedure employed did not show them. It is generally known that the composition and pH of a medium profoundly influence the relative growth rate and metabolism of most micro-organisms, and such factors may have contributed to the results obtained. However, insofar as the technique was standardised, the occurrence of differences may reflect real differences and similarities. In this case it would appear that the common root-surface fungi of both plants were similar, although the populations were not consistent between specimens of the same species and varied according to location, e.g., *T. viride* was absent from both species of plants at the Alexandra site. The incidence of *A. glauca* on roots of both species of tussock plants from the Alexandra site may account for the relatively high incidence of this fungus in screened plates. 'Other non-sporing' fungi were regularly isolated by the plating techniques employed. Season appeared to have an inconsistent effect on the more common fungi, although *Ceratostoma* sp. and *Penicillium novae-zelandiae* were isolated from plants of *F. novae-zelandiae* at all three sites only in spring.

GENERAL DISCUSSION

It has been suggested already that the soil fungal population of these perennial grasslands must be greatly influenced by the extensive root systems of the tussock and inter-tussock grasses. In fact there is probably not one portion of these undisturbed perennial-grassland soils which has not come under the influence of roots at some stage or other. Thus, broadly, the fungal flora may be regarded as being rhizospheric as the term 'rhizosphere' refers to that portion of the soil which is subject to the influence of the plant root system (Katznelson *et al.* 1948). However, the term rhizosphere, as generally applied in recent literature, refers more specifically to the zone of influence by living roots, and much work has been done on the effects of excretions and detritus from living roots upon soil micro-organisms.

In the present investigation a general similarity was noted between the more frequently isolated fungi from tussock soils and those fungi shown by Waid (1957) to be concerned in the succession of fungi decomposing ryegrass roots. The following fungi formed the greater proportion of both populations: *Rhizoctonia* spp., *Trichoderma viride*, *Cladosporium herbarum*, *Penicillium* spp., Mucorales (e.g. *Mucor hiemalis, Mortierella* spp., etc.), *Fusarium* spp. and sterile dark and hyaline hyphae. It is likely therefore that the active soil fungal population, as determined by screened immersion plates, is composed, mainly, of those fungi concerned in the decomposition of root materials and thus is not truly rhizospheric in the more restricted sense of the term.

With the great mass of roots in these soils the effects of the small amounts of excretions from the living roots are probably masked by the effects of the larger amounts of material from decomposing portions of roots. However these excretions, by nutritional or other effects, e.g., stimulating the germination of dormant spores (Jackson 1957), may have influenced the composition of the active fungal flora. These excretions are probably initially responsible for the development of a particular fungal population, which, later, will be influenced more by the products of root degradation rather than by root excretion. These influences may not necessarily involve any qualitative changes in the population and may only be reflected quantitatively, because it is well known that fungi are, physiologically, very adaptable (Foster 1949). This may account for the fact that in these grassland soils, where the fungal population must be greatly influenced by the grass root systems, results by the methods and sampling employed did not show any important qualitative differences between fungal populations of tussock and inter-tussock zones or of those close to, or at a distance from, tussock roots.

ACKNOWLEDGEMENTS

I wish to thank Dr. W. L. Gordon for identifying the species of *Fusarium* and Dr. A. Kerr for examining cultures of *Rhizoctonia* spp.

REFERENCES

BISBY, G. B.; TIMONIN, M. I.; JAMES N. 1935: Fungi Isolated from Soil Profiles in Manitoba. *Canad. J. Res. C13:* 47-65.

CHESTERS, C. G. C. 1948: A Contribution to the Study of Fungi in the Soil. *Trans. Brit. mycol. Soc. 30:* 100-17.

CHESTERS, C. G. C.; THORNTON, R. H. 1956: A Comparison of Techniques for Isolating Soil Fungi. *Ibid. 39:* 301-13.

EGGLETON, W. G. E. 1938: Influence of Environmental Factors on Numbers of Soil Micro-organisms. *Soil Sci. 46:* 351-63.

FOSTER, J. W. 1949: "Chemical Activities of Fungi." Academic Press, New York.

GARRETT, S. D. 1951: Ecological Groups of Fungi; a Survey of Substrate Relationships. *New Phytol. 50 (2):* 149-66.

HUGH, R.; LEIFSON, E. 1953: The Taxonomic Significance of Fermentative Versus Oxidative Metabolism of Carbohydrates by Various Gram-negative Bacteria. *J. Bact. 66:* 24-6.

JACKSON, R. M. 1957: Fungistasis as a Factor in the Rhizosphere Phenomenon. *Nature, Lond. 180:* 96-7.

JONES, P. C. T.; MOLLISON, J. E. 1948: A Technique for the Quantitative Estimation of Soil Micro-organisms. *J. gen. Microbiol. 11:* 54-69.

KATZNELSON, H.; LOCHHEAD, A. G.; TIMONIN, M. I. 1948: Soil Micro-organisms and the Rhizosphere. *Bot. Rev. 14 (9):* 543-587.

LODDER, J.; KREGER-VAN RIJ, N. J. W. 1952: "The Yeasts. A Taxonomic Study. North Holland Publishing Co., Amsterdam.

McLENNAN, E. I.; DUCKER, S. C. 1954: The Ecology of the Soil Fungi of an Australian Heathland. *Aust. J. Bot. 2 (2):* 220-45.

McMILLAN, C. 1956: Nature of the Plant Community. I. Uniform Garden and Light Period Studies of Fine Grass Taxa in Nebraska. *Ecology 37 (2):* 330-40.

ORPURT, P. A.; CURTIS, J. R. 1957: Soil Microfungi in relation to the Prairie Continuum in Wisconsin. *Ecology 38:* 628-37.

THORNTON, R. H. 1952: The Screened Immersion Plate: a Method of Isolating Soil Micro-organisms. *Research 5 (4):* 190-1.

——————1956a: *Rhizoctonia* in Natural Grassland Soils. *Nature, Lond. 177:* 230-1.

——————1956b: Fungi Occurring in Mixed Oakwood and Heath Soil Profiles. *Trans. Brit. mycol. Soc. 39:* 485-94.

——1958: Biological Studies of Some Tussock Grassland Soils. I. Introduction, Soils and Vegetation. *N.Z. J. Agric. Res. 1:* 913-21.

WAID, J. S. 1957: Distribution of Fungi Within the Decomposing Tissues of Ryegrass Roots. *Trans. Brit. mycol. Soc. 40:* 391-406.

WARCUP, J. H. 1950: The Soil Plate Method for Isolation of Fungi from Soil. *Nature, Lond. 166:* 117.

——————1951: The Ecology of Soil Fungi. *Trans. Brit. mycol. Soc. 34:* 376-99.

——————1955: On the Origin of Colonies of Fungi Developing on Soil Dilution. *Ibid. 38:* 298-301.

——1957: Studies on the Occurrence and Activity of Fungi in a Wheat Field Soil. *Ibid. 40:* 237-62.

WARREN, J. R. 1948: An Undescribed Species of *Papulaspora* Parasitic on *Rhizoctonia solani* Kuhn. *Mycologia 40:* 391-401.

APPENDIX

List of fungi isolated from any one of three tussock-grassland soils by screened immersion plate (SIP), soil plate (SP), and rhizosphere (Rh) and root (R) plating, techniques.

SIP	SP	Rh	R	
				PHYCOMYCETES
*	*	*		*?Pythium* spp.
*	*	*	*	*Mucor hiemalis* Wehmer
	*			*M. spinescens* Lendner
	*	*		*M. ramannianus* Moeller
	*			*M. plumbeus* Bonord
	*		*	*M.* nr. *sphaerosporus* Hagem
*	*	*	*	*Mucor* spp.
*	*	*	*	*Absidia glauca* Hagem
*	*	*	*	*Zygorhynchus moelleri* Vuill
*				*Cunninghamella* sp.
*	*	*	*	*Mortierella alpina* Peyronel
*	*	*	*	*M. elongata* Linm
*				*M. exigua* Linm
	*			*M. gracilis* Linm
*	*	*	*	*M. horticola* Linm
	*			*M. humilis* Linm
*	*	*	*	*M. minutissima* v. Tieghem
*	*			*M. nana* Linm
	*	*	*	*M. spinosa* Linm
*				*M. stylospora* Dix.-Stew.
				ASCOMYCETES
*	*	*	*	*Arachniotus* sp.
	*	*		*Pseudogymnoascus vinaceus* Raillo
				ASCOMYCETES (cont.)
	*		*	*Ceratostoma* sp.
*	*			*Chaetomium cochliodes* Palliser
	*			*C. globosum* Kunze
	*			*C. seminudum* Ames
	*	*		*Thielavia* sp.
				YEASTS
*	*	*		*Candida humicola* (Daszewska) Diddens et Lodder
*				*C. laurentii* (Kuff) Skinner
	*			*Cryptococcus diffluens* (Zach) Lodder et v. Rij
*				*C. terreus* di Menna
*	*	*		*Rhodotorula mucilaginosa* (Jorg.) Harrison
*				*Trichosporon cutaneum* (de Beurm., Gougerot et Vaucher) Ota
				FUNGI IMPERFECTI
*	*	*		*Phoma* sp.
*		*		*Phomopsis* sp.
*	*			*Coniothyrium* sp.
*				*Cryptomela sp.*
	*	*	*	*Pestalotia* sp.
*				*Streptomyces* spp.
*	*	*		*Cephalosporium acremonium* Cda.

FUNGI IMPERFECTI (cont.)

SIP	SP	Rh	R	
*	*	*		*C. asperum* Marchal.
*	*	*	*	*Trichoderma viride* Pers. ex Fr.
*	*		*	*Penicillium chrysogenum* Thom
	*	*	*	*P. citrinum* Thom
*	*	*		*P. corylophilum* Dierck
*	*	*		*P. cyclopium* Westl.
*				*P. expansum* (Link) Thom
*	*	*	*	*P. frequentans* Westl.
	*			*P.* sp. nr. *P. godlewski-P. resticulosum*
*	*	*	*	*P. janthinellum* Biourge
	*	*		*P. jenseni* Zaleski
		*		*P. lilacinum* Thom
	*	*		*P. lividum* Westl.
*				*P. luteum* Zukal
	*			*P. martensii* Biourge
	*			*P. melinii* Thom
*				*P. miczynskii* Zaleski
*	*	*	*	*P. nigricans* (Bain) Thom
*	*	*	*	*P. novae-zelandiae* v. Beyma
*				*P. palitans* Westl.
	*			*P. purpurescens* (Sopp) Raper et Thom
*				*P. rubrum* Stoll
*				*P. rugulosum* Thom
*	*	*	*	*P. soppii* Zaleski
*	*	*	*	*P. stoloniferum* Thom
*				*P. tardum* Thom
*				*P. terrestre* Jensen
*	*	*	*	*P. thomii* Maire
	*	*		*P. waksmani* Zaleski
*				*Penicillium* spp.
*		*		*Scopulariopsis* sp.
*				*Gliocladium roseum* Bain.
			*	*Paecilomyces* sp.
*				*Acladium* sp.
*	*			*Botrytis cinerea* Pers.
*	*			*Botrytis* spp.
*	*	*		*Verticillium* spp.
*	*			*Geomyces cretaceus* Traen
*	*	*		*Sporotrichum* sp.
*				*Dematium* sp.
	*	*	*	*Papularia sphaeronema* (Pers.) v. Hohn.
		*		?*Chalara* sp.
*	*	*		*Humicola grisea* Traen
	*	*		*Hormodendron hordei* Bruhne
*	*	*	*	*Hormodendron cladosporiodes* (Fr.) Sacc.
	*			*Oidiodendron griseum* Robak
*	*	*	*	*Cladosporium herbarum* Link
*				*Cladosporium* sp.
*	*	*	*	?*Torula* sp.
*	*	*		*Trichocladium* sp.
*				*Acrothecium* sp.
*	*	*	*	*Fusarium avenaceum* (Fr.) Sacc.
*	*	*	*	*F. sambucinum* Fuckel var. *coeruleum* Wr.
*	*	*	*	*F. oxysporum* Schlecht. emend Snyder et Hansen
*	*	*	*	*Cylindrocarpon radicicola* Wr.
*	*			*C. olidum* Wr.
*				*Rhizoctonia* sp.
			*	?*Chlamydosporus* sp.
*	*	*	*	Other non-sporing mycelia

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

III. YEASTS

By M. E. di Menna, Soil Bureau, Department of Scientific and Industrial Research, Wellington.

(*Received for publication, 19 June 1958*)

Summary

A qualitative examination was made of the yeasts in three series of samples of Omarama, Tekoa, and Taupo soils from areas of uncultivated tussock grassland at Alexandra, Bealey, and Waiouru. Thirteen species were isolated, but only five of these, *Cryptococcus albidus, C. diffluens, C. terreus, Candida humicola,* and *C. curvata,* occurred in important numbers. Except that *Candida curvata* was not found in the Omarama soil, all five species were common to the three soils, but a different species was dominant in each soil. *Candida humicola* was found almost exclusively in samples from the tussock zone. Genetic type, climate, and potential vegetation were different for each soil. It is not possible at this point to suggest which of these factors, if any, is responsible for the difference in species pattern.

INTRODUCTION

Very little information is available on yeast populations in soil. Many think that soil yeasts are uncommon and very probably are the result of contamination from vegetation (de Becze 1956). However, Miller and Webb (1954) in a report of a quantitative study of soil yeasts state: "It is questionable whether a group of organisms that can achieve even this limited abundance" (approximately 1,000 per gramme of soil) "in the highly competitive soil environment should be regarded as a negligible component of the soil flora, particularly since counts of 2,000 to 6,000 per gramme were not uncommon." An earlier piece of work by the present author (di Menna 1955) suggested that the soil yeasts were a distinct physiological group.

This paper records a qualitative examination of yeasts from the three tussock-grassland soils described by Thornton (1958).

MATERIALS AND METHODS

Yeasts were examined in the samples collected in the summer of 1955 of Omarama soil at Alexandra, Tekoa soil at Bealey, and Taupo soil at Waiouru. A site was chosen at each locality and in each site three pits were dug. Three samples were taken from each pit, one each from the T (tussock), I (inter-tussock) and S (subsoil) zones as defined by Thornton (1958). In all, cultures were made from twenty-seven samples of soil.

A little soil from each sample was suspended in sterile, 0.85% (w/v) saline and spread upon the surface of plates of modified Sabouraud (4% glucose, 1% peptone) agar which had been brought to a pH of 4 by the addition of 10% HCl to the molten medium immediately before the plates were poured. This medium suppresses all but acidophilic species of bacteria and supports the growth of almost all yeasts, the exceptions being almost entirely species that are parasitic on the animal body. Although bacteria do not grow at this low pH, some cells or microcysts must be able to survive, for yeast colonies subcultured directly to a solid medium of pH 6 frequently proved contaminated. A preliminary subculture in glucose yeast extract broth of pH 4 (Kurung 1942) usually gave pure yeast cultures.

The soil suspension cultures on Sabouraud agar plates were incubated at room temperature (*ca.* 18°C) for up to five days, when, if possible, twenty yeast colonies per sample were subcultured. Colonies were picked systematically from the plates in order to prevent the unconscious selection of large or otherwise conspicuous growths. In some cases, where the numbers of yeasts were small and spreading mould colonies great, it was impossible to recover the desired number of yeasts per sample. No yeasts could be recovered from one Taupo T-zone sample because of the presence of very many rapidly spreading moulds.

One hundred and seventy two yeasts were recovered from the nine Omarama soil samples, one hundred and fifty five from the Tekoa soil and one hundred and thirty nine from the Taupo soil. The isolates were identified by the criteria of Lodder and Kreger-van Rij (1952). *Pullularia pullulans* (De Bary et Low) Berkhout was treated as a yeast.

TABLE 1. YEASTS ISOLATED FROM THREE TUSSOCK-GRASSLAND SOILS

Zone *	Taupo				Tekoa				Omarama			
	T	I	S	Total	T	I	S	Total	T	I	S	Total
Debaryomyces kloeckeri Guill. et. Peju	—	—	—	—	—	—	—	—	—	—	2	*2*
Debaryomyces subglobosus (Zach) Lodder et v. Rij	—	—	—	—	—	—	—	—	—	—	15	*15*
Cryptococcus laurentii (Kuff.) Skinner	2	—	2	*4*	—	3	—	*3*	4	6	1	*11*
Cryptococcus albidus	2	25	9	*36*	3	11	1	*15*	—	—	1	*1*
Cryptococcus luteolus (Saito) Skinner	—	—	—	—	—	—	—	—	1	—	—	*1*
Cryptococcus diffluens	—	1	2	*3*	1	—	—	*1*	44	45	38	*127*
Cryptococcus terreus	11	29	19	*59*	21	25	6	*52*	1	1	1	*3*
Candida humicola	16	—	1	*17*	20	—	—	*20*	10	—	—	*10*
Candida scottii Diddens et Lodder	1	—	—	*1*	—	—	—	—	—	—	—	—
Candida curvata	5	1	4	*10*	15	18	30	*63*	—	—	—	—
Schizoblastosporion starkeyi-henricii	—	—	—	—	—	—	—	—	—	—	1	*1*
Trichosporon cutaneum (de Beurm., Gougerot et Vaucher) Ota	1	4	—	5	—	—	—	—	—	—	—	—
Pullularia pullulans	—	—	4	*4*	—	1	—	*1*	—	—	1	*1*
Total	38	60	41	*139*	60	58	37	*155*	60	52	60	*172*

* T tussock, I inter-tussock, S subsoil.

Results

Thirteen species were recovered; five of these, *Cryptococcus albidus* (Saito) Skinner, *C. diffluens* (Zach) Lodder et Kreger-van Rij, *C. terreus* di Menna, *Candida humicola* (Daszewska) Diddens et Lodder, and *C. curvata* (Diddens et Lodder) Lodder et Kreger-van Rij, made up 90% of the isolates. Except that *Candida curvata* was not found in the Omarama soil samples, all five species were common to the three soils. *Cryptococcus diffluens* was the dominant species in Omarama soil (73% of the isolates), *Candida curvata* in Tekoa soil (41%), and *Cryptococcus terreus* in Taupo soil (42%). *Cryptococcus terreus* also occurred in significant numbers in Tekoa soil (34% of total isolates), and *Cryptococcus albidus* made up 26% of the Taupo soil isolates. With the exception of one strain recovered from the S zone of Taupo soil, all isolates of *Candida humicola* were found in T-zone samples. The other four numerically important species were distributed throughout the T, I, and S zones.

Detailed results are given in Table 1.

Discussion

The distribution pattern of yeasts in the soils examined here resembles that of the majority of other groups of micro-organisms studied; there was some resemblance between the floras of the Taupo and Tekoa soils whilst that of the Omarama soil was quite distinct.

The soil yeasts encountered were similar in the taxonomically important characters, being imperfect, colourless, and non-fermenting, but it seems likely that properties not usually examined cause different species to flourish in different soils. *Cryptococcus diffluens* was dominant in the Omarama soil and *C. terreus* in the Taupo, with *C. albidus* occurring in lesser but still important numbers in the latter soil. All three species were present in both areas. In the laboratory, *C. diffluens* is separated from the other two species by its ability to assimilate sucrose but not lactose, *C. terreus* by its assimilation of both sugars. As it is unlikely that either of these two compounds is present in significant amounts in normal soils (Vallentyne 1957), it does not seem that ability to assimilate them could ever confer an advantage great enough to give one soil yeast dominance over another.

Characters that are probably of greater importance in determining distribution are the pH and temperature for maximum growth rate, ability to use the carbon sources present in plant debris, demand for mineral nutrients and growth factors, and lack of sensitivity to antibiotic substances produced by other members of the soil flora.

As there has been so little work upon soil yeasts, particularly in recent years when yeast taxonomy has been in a sufficiently advanced state to ensure unambiguous identifications, there are few overseas findings with which to compare the above results. Miller and Webb (1954) reported that few of the yeasts isolated from Canadian soils produced ascospores. Lund (1954), working with Danish soils, found *Cryptococcus albidus* in a number of samples, and *Schizoblastosporion* *starkeyi-henricii* Ciferri in a soil of pH 3.5 from a bog and in raw humus from a spruce forest. These two species were amongst those more rarely isolated in Lund's study, *Hansenula*, *Candida* (species other than *C. humicola* and *C. curvata*), and *Torulopsis* being the commonest genera. Capriotti (1955a), examining twelve samples of soil in the Netherlands, found *Saccharomyces*, *Hansenula*, *Pichia*, *Torulopsis*, and *Rhodotorula* spp. The yeast flora of some Italian soils examined by Capriotti (1955b) also showed no resemblance to the yeast flora of the New Zealand soils studied here.

Despite this, the species patterns in the three tussock soils were sufficiently well marked and consistent to suggest that a true yeast flora had been encountered. The results also strengthen a hypothesis put forward in an earlier piece of work (di Menna 1955), that the mineral soil yeasts are of a certain physiological type, colourless, capsulated, non-fermenting, capable of using nitrate as a nitrogen source, and able to synthesise a starch-like substance under suitable conditions. *Cryptococcus albidus*, *C. diffluens*, and *C. terreus* posses all these characters. *Candida humicola* and *C. curvata* are uncapsulated and cannot use nitrate nitrogen, but in other respects resemble the hypothetical type. It is possible that a sticky capsule is of value to a non-motile, non-mycelial, soil-inhabitaing organism, as by it the yeast cell may adhere to the surfaces of arthropods and worms and so be spread through the soil.

REFERENCES

BECZE, G. I. DE 1956: YEASTS: I, Morphology. *Appl. Microbiol. 4:* 1.

CAPRIOTTI, A. 1955a: Yeasts in Some Netherlands Soils. *Leeuwenhoek ned. Tijdschr. 21:* 145.

———1955b: I Lieviti di Alcuni Terreni dell'Italia Centrale. *Riv. Biol. 47:* 209.

KURUNG, J. M. 1942: The Isolation and Identification of Some Pathogenic Fungi from Sputum. *Amer. Rev. Tuberc. 46:* 365.

LODDER, J.; KREGER-VAN RIJ, N. J. W. 1952: "The Yeasts: A Taxonomic Survey." North-Holland Publishing Co., Amsterdam.

LUND, A. 1954: "Studies on the Ecology of Yeasts." Munksgaard, Copenhagen.

MENNA, M. E. DI 1955: A Search for Pathogenic Species of Yeasts in New Zealand Soils. *J. gen. Microbiol. 12:* 54.

MILLER, J. J.; WEBB, N. S. 1954: Isolation of Yeasts from Soil with the Aid of Acid, Rose Bengal, and Oxgall. *Soil Sci. 77:* 197.

THORNTON, R. H. 1958: Biological Studies of Some Tussock-grassland Soils. I. Introduction, Soils and Vegetation, *N.Z. J. agric. Res. 1:*

VALLENTYNE, J. R. 1957: The Molecular Nature of Organic Matter in Lakes and Oceans with Lesser Reference to Sewage and Terrestrial Soils. *J. Fish. Res. Bd Can. 14:* 33.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

IV. BACTERIA

By J. D. Stout, Soil Bureau, Department of Scientific and Industrial Research, Wellington.

(*Received for publication, 19 June 1958*)

Summary

A quantitative and qualitative study of the bacteria of three tussock-grassland soils has been made to determine the relative influence of vegetation and soil type on the flora.

Greatest numbers of bacteria tend to occur in the topsoil rather than in the subsoil and in the spring rather than the summer or autumn.

Of the five main taxonomic groups recognised, one (the Achromobacteriaceae) is considered to be chiefly derived from the leaf flora while the distribution of the aerogenic fermenters (Enterobacteriaceae and *Aeromonas*) is considered to be related to the relative organic content and the extent of the rooting systems in the three soils. The predominant flora consists chiefly of pseudomonads and is very similar in all three soils.

Generally, the flora consists of strongly oxidative and proteolytic strains, psychrophilic or mesophilic rather than thermophilic, requiring readily available sources of energy but not requiring growth factors and capable of using ammonium as a sole source of nitrogen. There is a positive correlation between the saccharolytic and proteolytic activities of the strains and denitrification.

Generally, it is considered that the activity of the flora is controlled primarily by the condition of the plant cover and particularly by root activity. Neither moisture nor temperature appear to be serious limiting factors.

Introduction

The present study of three areas of tussock grassland (previously described, Thornton 1958) was intended, firstly, to describe the nature of the microfauna and microflora of the soils and, secondly, to determine the relative influence of vegetation and soil type on the fauna and flora.

The three soils studied were derived from different parent materials, developed under different climates, and have had different vegetational histories. Consequently, they show differences in weathering and leaching and in their organic fraction. The three soils are: the Omarama soil near Alexandra, a fine sandy loam, high in exchangeable bases and with a very high base saturation, with a reduced and senile tussock cover; the Tekoa soil near Bealey, a silt loam of medium base status formerly covered by beech forest but now under tussock and introduced grasses; and the Taupo soil near Waiouru, a sandy silt of medium base status and with a vigorous plant cover of tussock and other grasses.

The study of the bacterial flora presented a formidable problem of identification but it became clear that determining the taxonomic

N.Z. J. agric. Res. 1: 943–957.

status of the different strains threw little light on the ecology of the soils, for different taxa commonly showed very similar physiological characteristics. For this reason in treating the bacterial flora the classification suggested by Hugh and Leifson (1953) has been used. This classification is based on the distinction between aerobic and anaerobic utilisation of sugars and the production, or not, of acid. Hugh and Leifson showed that these physiological differences were of taxonomic significance and both Leifson (1956) and Sneath (1956) have used these distinctions in re-classifying the genus *Chromobacterium*. It is clear, however, that the basic significance of the classification is ecological and that its chief taxonomic merit is that it affords a rational basis for a natural classification of bacterial taxa.

Four groups are distinguished.* Group A. Those that fail to produce acid aerobically or anaerobically from sugars. It is considered that those that use the sugars without acid production either assimilate them or oxidise them completely to carbon dioxide and water (Topley and Wilson 1948, p. 422). Group B. Those that produce acid aerobically but not anaerobically. The organic acids produced result from the incomplete oxidation of the sugars. Group C. Those that produce acid aerobically and anaerobically. Group D. Those that produce acid and gas both aerobically and anaerobically.

Biochemically the complete oxidation of the sugar molecule to carbon dioxide and water is the most efficient mechanism, but the less efficient oxidation to an organic acid is generally correlated with greater physiological activity, a higher rate of generation, and the ability to grow at lower oxygen tensions. Consequently, where there is ample food available Group B may have a competitive advantage relative to Group A, particularly where the oxygen tension is low, but, where the available food is restricted, the biochemically more efficient bacteria (Group A) will predominate. Similarly, the selective advantages of the fermenters (Groups C and D) will only be realised where there is a rich food source and where anaerobic conditions prevail for greater or lesser periods. Thus enteric bacteria are fermenters (Groups C and D) and fresh-water bacteria are non-fermenters (Groups A and B).

The flora of the present soils belongs predominantly to Group B and the fermentative part of the soil flora is associated with areas of increased root activity and concentrations of organic material. The character of this flora is considered to indicate an environment generally favourable for bacterial growth but subject at times to lowered oxygen tension and in the root region to short periods of anaerobiosis.

METHODS

Sampling and Counting

Sterile screw-capped 4-oz jars were used for sampling. Five samples from each zone (tussock (T) and inter-tussock (I) from the topsoil (2–4 in.) and S from the subsoil (12–14 in.)) were collected from each area, i.e. 15 samples in all from each soil site. The samples were dealt with as soon as possible after reaching the laboratory. Because of their geographical position, there was a greater delay with the South Island samples from Alexandra and Bealey, than with the North Island samples from Waiouru.

---

* The original nomenclature used for these groups by Hugh and Leifson (1953) has been found to be confusing. No better nomenclature, however, has been devised and for this reason the groups are distinguished in this paper simply by letters. Hugh and Leifson termed Group A, non-oxidisers, non-fermenters, Group B, oxidisers, non-fermenters, Group C, anaerogenic fermenters, Group D, aerogenic fermenters.

For each zone, sub-samples of 10 g of soil from each sample were bulked. To this bulked sample, 950 ml of sterile distilled water was added and this formed the primary dilution (1/20) for each zone. Further dilutions, usually of 1/2,000 and 1/200,000 were also made.

For plating, two media were employed. Difco Nutrient Agar, a standard medium for bacterial counts, and a dextrose-tryptone-yeast extract agar, hereafter referred to as DTY agar. It consisted of dextrose 5 g, Bacto-tryptone 4 g, Difco yeast extract 4 g, agar 15 g, distilled water 1 litre. Both media have a near-neutral reaction (pH 6.8).

For each dilution, at least four and often six plates were poured with both media. Plates were incubated at 25°c for two days before counting. As there is less profuse growth with nutrient agar than with DTY agar the nutrient-agar plates were generally easier to count but there was good agreement between the two series of plates.

Isolation and Identification

Representative colonies were picked off the plates and sub-cultured, a method of selection which resulted in a slight bias towards unique or unusual colonies. The DTY-agar plates, which favoured differentiation of the colonies and particularly chromogenesis, were more useful than the nutrient-agar plates for this purpose. Besides these two media growth of sub-cultures was also studied on potato dextrose agar, hereafter referred to as PDA. This consists of potato (200 g/litre) diced, steamed and filtered, dextrose (10 g/l) and agar (15 g/l), pH 6.8. Mannitol calcium glycero-phosphate agar (Hofer 1941) was found to be useful for testing the production of fluorescence.

As a routine, all sub-cultures were tested for their biochemical activities in the following media. Glucose and lactose broth: 1% sugar and 1% Bacto-tryptone with brom-thymol-blue (yellow pH 6.1, blue pH 7.7) as an indicator; sterilised by autoclaving at 15 lb for 15 minutes, the glucose being sterilised as a 10% solution and then distributed aseptically in the tubes of broth. Gelatin stabs: 12% commercial gelatin (Davis) sterilised by steaming for 20 minutes on three successive days, distributed in screw-capped McConkey bottles. Litmus milk: prepared from pasteurised milk and sterilised by steaming for 20 minutes on three successive days.

On the basis of their cultural characteristics, of their biochemical reactions in these media, of their morphology and Gram-stain reaction, 411 sub-cultures were sorted into a number of groups. From each of these groups a number of representative strains were selected for more detailed study. A further range of biochemical activities was tested including indole production, the Voges-Proskauer and methyl red tests, urease activity (Christensen's medium), the production of sulphide, starch hydrolysis, cellulose decomposition, denitrification, and citrate and nitrate utilisation. The methods employed generally followed those described in the Manual of the American Bacteriological Society except that the modification of the Voges-Proskauer test suggested by Fouad and Richards (1953) was found preferable, and for the *Bacillus* group the media suggested by Smith, Gordon, and Clark (1952) were used. Ammonification was tested by adding Nessler's reagent to a peptone broth culture after 7 and 14 days' culture. A lead acetate paper in the neck of the tube tested sulphide production.

The majority of strains were able to grow in a basal salt solution consisting of: $(NH_4)H_2PO_4$, 1.0 g; KCl, 0.2 g; $MgSO_4.7H_2O$, 0.2 g; dist. water, 1 litre (adjusted to pH 7.0 with N NaOH) with 1% carbohydrate. This medium was sterilised either by autoclaving or by Seitz filter and brom-cresol purple (yellow pH 5.4—purple pH 7.0) used as an indicator. Tubes were inoculated from slopes and the ability to grow in this medium was assumed to indicate the absence of any growth-factor requirements. The final pH of some of these carbohydrate cultures was checked electrometrically.

Anaerobic growth and fermentation were checked by use of a McIntosh and Fildes jar.

In the *Nocardia* group, strains were tested for their ability to use paraffin. Liquid paraffin was added aseptically to basal salt-agar slopes and the growth on these slopes compared with controls without paraffin.

Pectin (1% Hopkins and Williams, Grade 40) in basal salt solution with brom-cresol purple as an indicator was used to test pectin utilisation and pectinolytic activity.

Flagellation of 25 selected strains was determined by electron micrographs prepared by W. S. Bertaud of the Dominion Physical Laboratory.

RESULTS

Numbers of Bacteria in the Three Soils

The mean plate counts for the three different zones for the seasons sampled are given in Table 1.

TABLE 1. NUMBERS OF BACTERIA (10^6/g) IN THE THREE SOILS

	Zones	Spring 1953	Summer 1954	Summer 1955	Autumn 1954
Taupo soil (Waiouru)	T	128	0.56	0.44	0.36
	I	4.8	0.11	0.07	0.18
	S	4.6	0.05	0.04	0.06
Tekoa soil (Bealey)	T	100	0.16	0.28	0.07
	I	80	0.31	0.36	0.05
	S	320	0.08	0.09	0.02
Omarama soil (Alexandra)	T	16	0.9	1.0	0.12
	I	160	0.8	0.9	0.06
	S	32	0.16	0.09	0.02

The plate counts for the three areas studied were all of the same order for each season. The spring counts were significantly higher than the summer and autumn counts, and, except for the spring counts, the subsoil counts were significantly lower than the topsoil counts (tussock and inter-tussock). Also, except for the spring counts, the average tussock zone counts were higher than the inter-tussock counts. The individual exceptions occurred with counts of the Omarama and Tekoa soils and may have been due to population changes following the longer delay in plating these South Island samples.

The three factors commonly affecting bacterial populations are available food, moisture, and temperature. The results suggest: that the supply of bacterial nutrients is similar for the three soils despite the differences in plant cover and total carbon status as the counts are all of the same order; that the most readily available nutrients are in the topsoil and probably derive from root excretions and freshly sloughed root caps and epidermis: that neither moisture nor temperature differences in the three seasons, which are dissimilar for each of the soils, determine the population but that the spring peak may be due to the higher moisture content consequent on the thawing of the soil which in winter is frozen for several months at Alexandra, snow-covered at Bealey and may also be temporarily frozen or snow-covered at Waiouru.

Taxonomic Character of the Bacterial Flora in the Three Soils

The nature of the flora isolated is a reflection of the methods and media employed. The dilution technique, the enriched media and aerobic culture result in the growth of the predominantly aerobic, aquatic and heterotrophic bacteria without any special growth requirements and tolerant of intense competition. The dominant flora so isolated consists of pseudomonads. Only one type, mentioned later, failed to grow on serial transplant on the ordinary laboratory media, i.e. nutrient agar and DTY agar. Moreover, the great majority of strains, excepting *Bacillus* strains, were able to grow in the basal salt medium with a carbohydrate source. This is evidence that the flora is predominantly non-exacting in its growth-factor requirements, differing in this respect from the flora studied by Lockhead and others (Lockhead and Burton 1957; Payne *et al.* 1957).

Despite the bulking of samples, the same soil can give considerable variation by the plating technique. Consequently no great significance can be attached to apparent seasonal differences, or to the zone differences which seem to be indicated in Table 2. It will be seen that in many cases where 10 or less strains of a type have been isolated they have been isolated from the subsoil plates, whereas when more than ten strains have been isolated they have come chiefly from topsoil plates. It seems possible that the drop in total population in the subsoil zone has favoured the less competitive strains, which are not swamped by the growth of the pseudomonads. Conversely, the taxa isolated predominantly from the topsoil plates probably represent the dominant soil flora.

Overall, the flora is very similar for the three soils. It is possible to distinguish a primary autochthonous flora, principally achromogenic

TABLE 2.

Bacterial Type		Chromogenesis	Motility	Carbohydrate Metabolism			Protein Meta-	
				Glucose	Sucrose	Lactose	Gelatin	Litmus Milk
Pseudomonas fluorescens	I	N. Yellow-blue-green D. Brown	+	A or Alk	Alk	Alk	+	A PR
Pseudomonas fluorescens	II	N. Blue-green	+	A or Alk	Alk	Alk	+	A CR
Pseudomonas	III		+	A or Alk	Alk	Alk	+	A
Pseudomonas	IV	D. Dark brown	+	A	Alk	Alk	+	A
Pseudomonas chlororaphis	V	D. Yellow N. Green	+	A	Alk	Alk	+	A R
Pseudomonas	VI		+	A	Alk	Alk	+	A P
Xanthomonas		N. Yellow	+	A	A	A	−	A R
Aeromonas		D. Yellow fluorescent	+	A & G	A & G	A & G	+	A PR
Achromobacter			+	Alk	Alk	Alk	±	Alk
Flavobacterium	I	N. Orange	−	A or Alk	Alk	Alk	+	A R
Flavobacterium	II	N. Yellow	+	Alk	Alk	Alk	−	Alk
Chromobacterium		N. Violet	+	A or Alk	Alk	Alk	+	A R or Alk R
Serratia		N. Red	+	A & G	A & G	A & G	+	A R
Aerobacter cloacae			+	A & G	A & G	A & G	+	A R
? *Proteus*			+	A & G	A & G	A	+	A
Bacillus cereus			+	A	A	Alk	+	A PR
Bacillus mycoides			+	A	A	Alk	+	A PR
Bacillus megaterium			−	Alk	Alk	Alk	+	Alk PR
Nocardia corallina	I	N. Pink	−	Alk	Alk	Alk	−	Alk
Nocardia	II	N. Red	−	Alk	Alk	Alk	−	Alk
Nocardia	III	N. Yellow and Orange	−	Alk	Alk	Alk	+	Alk
Nocardia	IV	N. Violet	−	Alk	Alk	Alk	−	Alk
Nocardia	V		−	Alk	Alk	Alk	−	Alk
? *Myxococcus*		N. Blue and Orange	−	A		A	+	A PCR

Chromogenesis: on DTY agar. N—non-diffusable. D—diffusable.

Carbohydrate metabolism: Glucose, Sucrose, Lactose: 1% sugar, 1% Bacto-tryptone, bromthymol-blue: A, acid; A & G, acid and gas; Alk, alkaline.

Gelatin: Liquefaction +, non-liquefaction −, strain variation ±.

Litmus Milk: P, peptonized; C, clotted; R, reduced; A, acid; Alk, alkaline.

Sulphide production: Blackening of lead acetate paper over peptone broth +, strain variation ±.

Ammonification: Nessler's reaction, orange +, brown + +

Denitrification: Zobel's medium. NO_2, reduction to nitrite; N_2, reduction to nitrogen.

Nitrate utilization: Glucose, basal salts with KNO_3; growth +, no growth −, strain variation ±.

bolism		Denitrification		Nitrate Utilization	Temperature Growth Range			Glucose Metabolism	Growth Factor Requirements	Ecology	Distribution						No. Strains Tested
Production of											Sites			Zones			
H_2S	NH_3	NO_3	NO_2		2°	25°	27°				O	Te	Ta	T	I	S	
		—	—	—	+ +	+ + +	—	O	—	S	—	+	+	2	17	7	26
		—			+ +	+ + +	—	O	—	S	+	—	—	2	5	2	16
—	—	NO_2	—	±	+ +	+ + +	—	O	—	S	+	+	+				
—	+	N_2	N_2	+	+ +	+ + +	+ ±	O	—	S	+	—	+	4	4	3	20
—	+	NO_2	—	—	+ +	+ + +	—	O	—	S	+	—	—	0	0	6	6
—	—	—	—	+	+ +	+ + +	—	O	—	S	—	—	+	0	1	9	10
	—	NO_2	—	—	—	+ + +	—	O	—	S	—	+	—	1	1	0	2
—	+	NO_2	N_2	+	+ +	+ + +	+ + +	F	—	S	—	—	+		1		1
±	—	NO_2	—	±	—	+ + +	—	O or N-o	—	S	+	+	+	6	6	2	14
+	+	—	—	—	+	+ + +	—	O	+	S, L	+	+	+	12	3		17
±	—	NO_2	—	—	—	+ + +	+ +	O	—	S, L	+	+	—	2	2	4	9
—	+ +	NO_2	N_2	—	+ +	+ + +	—	O	—	S, L	+	+	+	2	1		3
+	—	NO_2	N_2	—	+ +	+ + +	+ + +	F	—	S	—	+	+	7	5	2	18
+	—	NO_2	N_2	—	+ +	+ + +	+ + +	F	—	S, L	+	+	+	12	5	5	24
—	—	NO_2	N_2		+	+ + +	+ +	F	+	S	—	—	+		1		1
—	+	NO_2	N_2	—	—	+ +	—	F	+	S	+	+	+	5	12	4	21
	+	N_2	—	—	—	+ +	—	F	+	S	+	+	+				
—	—	NO_2	—	—	—	+ +	+ + +	N-o	—	S	+	+	+	7	3	3	13
	—			—	∓	+ +	—	N-o	—	A	+	+	+	1		3	4
—	—	NO_2	—	—	—	+ +	+	N-o	—	S, A	—	+	—		1		1
+	+	NO_2	—	—	+	+ +	+	N-o	+	S	+	+	—	2	1		3
	—				—	+ +	—	N-o	—	S	—	+	—		1		1
	+	N_2	—	—	—	+ +	—	N-o	—	S	—	+	+	1	1		2
	—		—		—	+ +	—	O	+	S, Litter	—	+	—		3		3
														64	71	57	215

Temperature growth range: on DTY agar slopes: poor growth +, fair growth + +, good growth + + +, strain variation ±.

Glucose metabolism: 1% sugar, basal salts, brom-cresol-purple.

N-o, non-oxidative—no acid produced; O, oxidative—acid produced aerobically; F, fermentative—acid produced aerobically and anaerobically.

Growth factor requirements: —, able to grow in basal salts with carbohydrate and ammonium nitrogen; +, not able to grow in basal salts with carbohydrate and ammonium nitrogen.

Ecology: S—soil, A—airborne, Litter—forest litter, L—leaf.

Distribution O, Omarama soil; Te, Tekoa soil; Ta, Taupo soil; T, tussock; I, inter-tussock; S, subsoil.

and consisting essentially of *Pseudomonas*, *Bacillus*, *Aerobacter*, and *Nocardia:* a supplementary autochthonous flora, including *Serratia* and *Aeromonas*, occurring only in soils with more extensive root infiltration; and an epiphytic flora, consisting principally of chromogens, such as *Flavobacterium*, commonly associated with leaves, and which, with the spore-formers, form the bulk of the air-borne flora (unpublished work). In these three soils the primary autochthonous flora is very similar, with some differences apparent in the Omarama soil, the supplementary flora associated with each area shows further differences in the broad pattern, while the epiphytic types occurring are similar because of the similarity of the epiphytic flora of the plant cover.

Taxonomically the bacterial flora falls into five main groups. Some of the biochemical characteristics of the taxa are listed in Table 2.

1. PSEUDOMONADACEAE. The great majority of strains were pseudomonads. This is partly to be expected from the methods and media employed, but it also indicates that they are the most numerous of the aquatic bacteria in these soils. Most of the strains belong to the *Pseudomonas fluorescens* group. The characteristic blue-green pigmented strains were regularly present in all three soils throughout the three seasons, although sometimes absent from the subsoil plates. The Omarama strains differed from those present in the other two soils in generally failing to produce browning of the agar in old cultures. Initially also, many of the Omarama strains failed to produce acid in glucose broth. The majority of pseudomonads were non-pigmented but biochemically indistinguishable from *Ps. fluorescens*. A third group biochemically similar to *Ps. fluorescens* apart from denitrification and ammonification produced a very dark browning of the agar.

Culturally distinct from the *Ps. fluorescens* group were two strains of yellow chromogens, similar to *Xanthomonas*, but with lophotrichate flagellation. Six strains of a very characteristic type, *Ps. chlororaphis*, which produces a yellow water-soluble pigment and a non-diffusable green pigment, were isolated from the Omarama soil. A non-pigmented type, producing characteristic fluorescence on mannitol calcium glycerophosphate agar was isolated only from the Taupo soil. This type produced characteristic pin-point colonies within the agar on DTY slopes and biochemically was able to produce acid from lactose in the basal salt medium.

2. ACHROMOBACTERIACEAE. The Achromobacteriaceae include the genera *Achromobacter* and *Flavobacterium*. *Chromobacterium*, at present included in the Rhizobiaceae, is for convenience grouped with them. They are essentially epiphytic in origin (unpublished work).

Two types of *Flavobacterium* were isolated. The most common was a non-motile orange chromogen present in all three soils and occurring in the topsoil in all seasons. The second type was a motile yellow chromogen recorded only from the Omarama and Tekoa soils.

Chromobacterium occurred in all three soils but only three strains were isolated. These strains produced acid from lactose in the basal salt medium.

A number of non-pigmented peritrichous strains were isolated from all three soils. Typically they failed to produce acid from glucose in the broth cultures, although some strains produced acid in the basal salt medium; only a few strains liquefied gelatin and all turned litmus milk alkaline. More than one taxon is probably involved but they all belong to the *Achromobacter-Alcaligenes* group and so have been referred to the former genus (cf. Topley and Wilson 1948).

3. Enterobacteriaceae. The Enterobacteriacae include saprophytic representatives of this family. For convenience I have included here an *Aeromonas* which is, of course, a member of the Pseudomonadaceae.

Aerobacter cloacae was present in all three soils. *Serratia* was present in the Tekoa and Taupo soils but was much more common in the Taupo soil. A strain of *Aeromonas* was also isolated from the Taupo soil as well as an unidentified strain of aerogenic fermenter, nutritionally exacting but not satisfied with nicotinic acid and failing to produce gas from lactose although fermenting glucose with the production of acid and gas.

The incidence of these fermenters in the three soils is correlated with the organic content of the soils measured by the total carbon figures which, for the topsoils of Omarama, Tekoa and Taupo, are 3%, 5.5%, and 6% respectively. These figures relate to the degree that roots penetrate the soil which in turn is correlated with the extent of plant cover and the vigour of the tussock growth. The absence of *Serratia* from the Omarama soil and of *Aeromonas* and the unidentified type from the Omarama and Tekoa soils may therefore be attributed to these differences in vegetation. The fermenters appear to be a rhizophilic group, presumably since the tussock zone may afford those conditions of rich nutrients and partial anaerobiosis which give the group its selective advantages (cf. Clements 1921). These conditions appear to be most characteristic of the Taupo soil which has the greatest proportion of fermenters (see Table 3) and also the greatest number of fermentative taxa.

4. Bacillaceae. The spore-forming Bacillaceae were represented in all three soils and at all seasons by *Bacillus cereus* and *Bacillus mycoides* although only a few strains of the latter were isolated for confirmatory tests as the colonial form is so characteristic. They formed, however, a relatively common group. The other species commonly present in all three soils was *B. megaterium*.

On PDA plates inoculated from a suspension of the Taupo soil which had been heated in a water bath for 5 minutes at 80°c, the *Bacillus cereus-Bacillus mycoides* group still proved to be the dominant species. *B. cereus* occurred on 7 of the 8 plates and *B. mycoides* on 3, but *B. polymyxa* also occurred on 3 plates and *B. pantothenticus* on 1. Curiously, *B. megaterium* was altogether absent from these plates. In my experience the *B. megaterium* strains isolated have not proved as actively sporogenous as the *B. cereus* group which may account for their absence.

On the other hand, of 12 isolates from dilution plates prepared from the Omarama soil with a very weak soil extract agar (of

Omarama soil) 6 proved to be *B. megaterium,* one was a yeast *Cryptococcus diffluens,* one a *Nocardia,* and four Gram-negative rods, three of which were probably achromogenic *Pseudomonas* and one an *Achromobacter.*

These two variations in technique reveal the cultural differences of the different groups today but they do not indicate that any major element in the fauna has been omitted through using a dilution technique and an enriched medium.

5. ACTINOMYCETACEAE. Several types of *Nocardia* were isolated from these soils although only a few strains of each type. Of those showing definite branching, four types were chromogenic being pink, red, violet, and yellow, and two strains failed to produce a pigment. Of these, all except the red strains were able to use paraffin as a carbon source. Representative strains of all these types were isolated from Bealey but apart from the pink chromogen (*N. corallina*) were not common in any area.

Besides these five main groups there were a number of strains which could not be classified within these taxa. A single *Micrococcus* strain was isolated from the Taupo soil. The absence otherwise of true cocci was remarkable, since they are commonly reported from agricultural soils. Coccoid stages of *Nocardia* are, however, often confused with true *Micrococcus* strains. A few strains of an unidentified bacterium which failed to grow on the second transplant were isolated from the Taupo soil. This minute motile, non-sporing, Gram-negative bacillus, perhaps a pseudomonad, failed to produce acid from glucose in broth culture but slowly liquefied gelatin. It has also been isolated from peat from one of the subantarctic islands (Auckland Is.) which suggests that it may be associated with the accumulation of organic matter in the Taupo soil (Bailey 1953). Three strains of a type of Myxococcaceae forming simple fruiting bodies were isolated from the Tekoa soil.

TABLE 3. PHYSIOLOGY OF SOIL BACTERIA

Present Classification	Classification of Hugh and Leifson	Taupo soil %	Tekoa soil %	Omarama soil %	
Group A	Non-oxidisers non-fermenters	25	15	20	Aerobes
Group B	Oxidisers non-fermenters	53	70	62	
Group C	Anaerogenic fermenters	5	5	7	Facultative anaerobes
Group D	Aerogenic fermenters	17	10	11	

Classification of 411 strains from the three soils according to the scheme of Hugh and Leifson (1953), based on acid production in glucose-tryptone broth with brom-thymol blue as indicator.

An attempt to evalute anaerobic activity of the heterotrophic flora was made by adding to a few grammes of soil in a test tube about 10 ml of DTY agar. The tubes were placed in a sealed desiccator which was evacuated, flushed, and then filled with nitrogen. Growth which resulted in the fracturing of the agar occurred with the topsoil from all three areas and with the subsoil of Omarama and Tekoa soils after a week at room temperature.

Considered taxonomically, the floral pattern is not perhaps of great significance for the flora consists of taxa widely distributed in nature, with few exceptions such as *Nocardia*. It is possible, however, to classify the strains physiologically according to the scheme of Hugh and Leifson (1953). A classification of the strains isolated from the three soils on this basis is given in Table 3. This classification is based on the initial reaction of the strains in dextrose-tryptone broth with brom-thymol blue as the indicator. Weak acid producers in this medium fail to give an acid reaction which is obtained in the basal salt medium. As shown in Table 3, the four categories are similar for the three soils. The majority of strains produce acid aerobically but not anaerobically and there are then roughly equal proportions of non-acid producers and fermenters. With the present strains, denitrification and liquefaction of gelatin correlate positively with acid production, so that this classification gives a measure of the overall biochemical activity of the strains. On the other hand, the ability to use phenol and paraffin is associated with the absence of acid production, e.g. *Nocardia*. In Fig. 1 the data from all three soils are condensed diagrammatically to show the saccharolytic and proteolytic grouping of the strains in

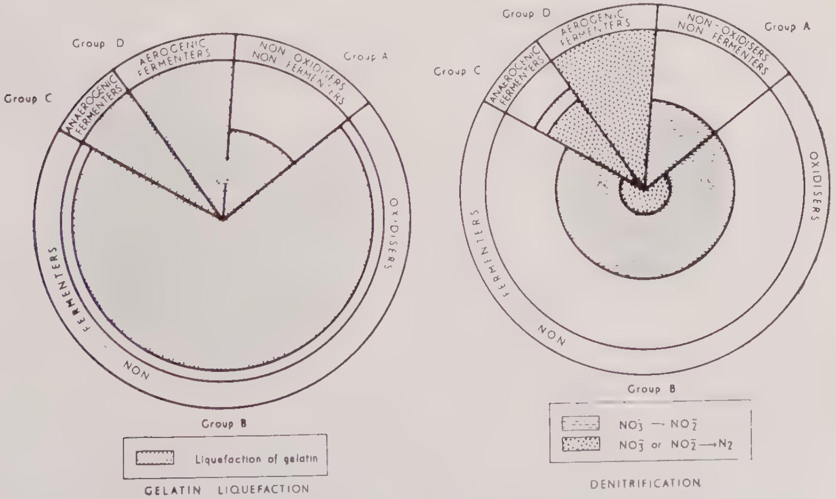


Fig. 1.—Analysis of the glucose metabolism (glucose-tryptone-brom-thymol-blue), gelatin liquefaction, and denitrification of 411 strains isolated from the three soils. The primary classification after Hugh and Leifson (1953). Group A, no acid from glucose broth; Group B, acid produced aerobically; Group C, acid produced aerobically and anaerobically; Group D, acid and gas produced aerobically and anaerobically.

relation to the different taxa. These diagrams of the overall character of the flora of grassland soil may be contrasted with the results of Taylor (1942) with lake water and of Thomas and Thomas (1955) with farm water supplies. Biochemically the soil flora is more active than the lake water flora but is very similar to the flora recorded by Thomas and Thomas. The major difference between soil and water is, however, the very much higher counts obtained from soil dilutions, commonly 10^6/g or more, compared with water counts, generally less than 10^3/ml.

Role in the Soil Organic Cycle

Carbon. With the exception of the *Nocardia* strains, which were able to use paraffin and phenol as a carbon source, the bacteria isolated required a readily accessible source of energy. None of the strains tested were able to attack filter paper* and although the great majority were able to use pectin (Hopkins and Williams, Grade 40) no strain was sufficiently pectinolytic to destroy the gelation of this medium. Starch was broken down most strongly by the ephiphytic and rhizophilic strains. The widest range of simple carbohydrates was used by the fermenters (Groups C and D), less by the incomplete oxidisers (Group B), and least by Group A. The range of fermentation products, such as acetyl-methyl-carbinol, showed a similar relationship.

Nitrogen. The great majority of strains were able to use ammonia as a nitrogen source but relatively few were able to use nitrate. A number of types, including all the fermenters (Groups C and D), were able to use nitrate as an oxygen source, denitrifying nitrate to nitrite or nitrogen, and also nitrite to nitrogen in some cases. Consequently, there was a greater tendency for nitrate-nitrogen to be lost by denitrification than for it to be synthesised into organic nitrogen. A number of strains were found to be urease positive. This is not a taxonomic character but it is reasonable to assume that any urea, from other soil organisms such as earthworms or ciliate protozoa, or from rabbits or grazing stock, would be dissimilated into ammonia. Apart from autolysis, this probably provides the chief surplus of ammonia, for none of the autochthonous soil bacteria are strong ammonifiers as compared with the epiphytic types such as *Chromobacterium,* and almost all can synthesise it into organic nitrogen.

Sulphur. No study was made of the metabolism of inorganic sulphur, but several types, particularly *Flavobacterium, Serratia,* and *Aeromonas,* produced sulphide from peptone.

Generally, in regard to the organic cycle, the bacteria fall into three main groups: the saprophytes, the scavengers, and the epiphytic types derived from the leaves. Biochemically the epiphytic types tend to be weak acid producers but to release ammonia and hydrogen sulphide from proteolysis. Typically these characteristics are associated with a rich supply of nutrients on the leaf surface and a high oxygen tension. The scavengers, which tend to be isolated on nutritively very poor media, such as soil extract agar, are distinguished by their ability to attack less readily available carbon sources, by their complete oxidation of sugars, by their lessened proteolytic activity, and by their slow growth in a limited temperature range. The saprophytes fall into two groups, both physiologically very active over a wide temperature range, actively proteolytic, and incomplete oxidisers of carbohydrates. They are dependent therefore upon a more-than-sufficient supply of simple carbohydrates for growth. One group, the pseudomonads, are aerobic, and the second, the aerogenic fermenters, are facultatively anaerobic and consequently have a selective advantage where anaerobic conditions are likely to be associated with a nutritively rich environment, i.e. the root surface. It seems likely that both these groups depend upon soil conditions being generally very favourable for bacterial growth and their numbers are much more susceptible to fluctuations than those of the scavengers. On the whole, the saprophytes will compete with other soil organisms for the available nutrients. Occasionally, as in denitrification, their activities are detrimental, sometimes, as perhaps in the breakdown of urea, favourable; but, generally, their role is to store in their cells a large part of the readily accessible organic nutrients constituting the soil organic cycle.

---

* A unique strain of *Pseudomonas* isolated from Bealey and forwarded to Dr. Haynes, was found by him to decompose cellulose slowly. He has provisionally identified it as a strain of *Ps. effusa.*

Discussion and Conclusions

Recently, van Niel (1955) has suggested that there are two ecologically distinct soil floras: an aquatic flora, predominantly pseudomonads, and a strictly terrestial flora, comprising *Nocardia* and *Mycobacterium*. He further suggested that, despite their predominance in aquatic conditions, the pseudomonads 'do not constitute a quantitatively significant proportion of the soil microflora' (van Niel 1955, p. 214). The present study suggests that for these grassland soils van Niel's generalisation does not hold. Pseudomonads appear to be the major constituent of the aerobic heterotrophic flora.

There are a number of reasons for considering that the present study is not misleading in this respect. First, the pseudomonad flora predominates in all three soils despite the very great variation in moisture status (Omarama soil 12–29%; Tekoa soil 31–58%; Taupo soil 37–62%) and annual rainfall (Omarama soil site, about 20 inches; Tekoa soil site, about 65 inches; Taupo soil site, about 48 inches). Further, the bacterial counts are all of the same order for the three soils and are determined principally by the pseudomonads. Secondly, since the pseudomonads predominate on the plates, the spring peak of 1953 was due to a marked increase in the pseudomonad population which is hardly compatible with the view that they do not constitute a quantitatively significant proportion of the flora. Thirdly, the view that the aquatic phase in soils is of less significance microbiologically than the non-aquatic phase is not compatible with the presence in soil of a large and strictly aquatic microfauna.

From these considerations it seems safe to conclude that the bacterial flora is not limited by moisture. Further, since there is no marked rise in summer, temperature is not a limiting factor. This suggests that the flora, which is dependent upon readily available nutrients, is controlled primarily by the activity of the plant cover and particularly their roots. So at the Taupo soil site, which has the most dense and complete ground cover with the most extensive rooting system, there occurs the greatest proportion of fermenters with the regular appearance of *Serratia* as well as two other types not recorded from the other two soils. At the Tekoa soil site, where the cover is less dense, *Serratia* still occurs, but less frequently. At the Omarama soil site, where the plant cover is relatively poor, the only fermenter is *Aerobacter*. It is difficult to distinguish the relative importance of the luxuriance of plant and root growth and the accumulation of organic matter (measured as total carbon) in the soil in so affecting the floral pattern, for in the present instance the two variables are positively correlated, but subsequent work has confirmed the association of the fermenters with roots.

Differences due to other soil factors are less evident. Indeed the close resemblance of the three microfloras is very striking. The small differences between the strains of the chromogenic *Ps. fluorescens* isolated from Omarama soil and the strains isolated from the other two soils and the isolation of the very characteristic *Pseudomonas chlororaphis* from Omarama soil but not from Tekoa or Taupo soils suggest that other soil factors may play a selective role in determining, within a particular physiological and ecological group, the taxa present in different soil types.

ACKNOWLEDGEMENTS

I am indebted to B. L. Stuart, Miss C. Arbon and Mrs. E. Guy for technical assistance during the course of this work.

I am also indebted to Dr. William C. Haynes, of the U.S. Department of Agriculture, Agricultural Research Service, Peoria, Illinois, for identifying the strains of *Ps. chlororaphis*.

REFERENCES

BAILEY, R. W. 1953: Composition of the Soil-Organic-Matter Complex in Pumice Soils of the Taupo Ash Shower. *N.Z. J. Sci. Tech. A34:* 415-20.

CLEMENTS, F. E. 1921: Aeration and Air-Content. The Role of Oxygen in Root Activity. *Carneg. Instn Publ. 315.* 185 pp.

DOWSON, W. J. 1949: "Manual of Bacterial Plant Diseases". A. & C. Black, London.

FOUAD, M. T. A.; RICHARDS, T. 1953: The Use of Glucose Inorganic Salts Media in Classification of the Coli-Aerogenes Bacteria. I. The Methyl Red and Voges-Proskauer Reactions. *Proc. Soc. appl. Bact. 16:* 35-44.

HOFER, A. W. 1941: A Characterization of *Bacterium radiobacter* (Beijerinck and van Delden) Löhnis. *J. Bact. 41:* 193-224.

HUGH, R.; LEIFSON, E. 1953: The Taxonomic Significance of Fermentative versus Oxidative Metabolism of Carbohydrates by Various Gram-negative Bacteria. *J. Bact. 66:* 24-6.

LEIFSON, E. 1956: Morphological and Physiological Characteristics of the Genus *Chromobacterium. J. Bact. 71:* 393-400.

LOCKHEAD, A. G.; BURTON, M. O. 1957: Qualitative Studies of Soil Microorganisms. XIV. Specific Vitamin Requirements of the Predominant Bacterial Flora. *Canad. J. Microbiol. 3:* 35-42.

Niel, C. B. Van 1955: Natural Selection in the Microbial World. The Second Marjory Stephenson Memorial Lecture. *J. gen. Microbiol. 13:* 201-217.

Payne, T. M. B.; Rouatt, J. W.; Lockhead, A. G. 1957: The Relationship between Soil Bacteria with Simple Nutritional Requirements and Those Requiring Amino-Acids. *Canad. J. Microbiol. 3:* 73-80.

Smith, N. R.; Gordon, R. E.; Clark, F. E. 1952: Aerobic Spore-forming Bacteria. *U.S. Dep. Agric. agric. Monogr. 16.*

Sneath, P. H. A. 1956: Cultural and Biochemical Characters of the Genus *Chromobacterium. J. gen. Microbiol. 15:* 70-98.

Taylor, C. B. 1942: Bacteriology of Fresh Water. III. The Types of Bacteria Present in Lakes and Streams and Their Relationship to the Bacterial Flora of Soil. *J. Hyg. 42:* 284-96.

Thomas, S. B.; Thomas, B. F. 1955: The Bacteriology of Farm Water Supplies: a Study of the Colony Count in 72 hours at 22°. *J. appl. Bact. 18:* 312-21.

Thornton, R. H. 1958: Biological Studies of Some Tussock-grassland Soils. I. Introduction, Soils and Vegetation. *N.Z. J. agric. Res. 1:* 913-21.

Topley and Wilson's "Principles of Bacteriology and Immunology." 3rd ed. 1948. Edward Arnold, London.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

V. NON-SYMBIOTIC NITROGEN-FIXING BACTERIA

By D. J. Ross, Soil Bureau, Department of Scientific and
Industrial Research, Wellington.

(Received for publication, 19 June 1958)

Summary

Three New Zealand tussock-grassland soils, viz Taupo soil near Waiouru, Tekoa soil near Bealey, and Omarama soil near Alexandra, have been found to contain the nitrogen-fixing anaerobic bacterium *Clostridium butyricum*, in numbers comparable with those reported in soils in other temperate countries. There is no marked variation in the numbers of this *Clostridium* in the different soils and there is no consistent difference between samples taken from the base of *Festuca* tussock plants or between well-spaced *Festuca* tussock plants.

Azotobacter spp. were not detected. Other aerobic bacteria and yeasts isolated from the soils or from leaves of *Festuca* tussock plants have been found to be without appreciable nitrogen-fixing powers.

It is suggested that the contribution of non-symbiotic bacteria to the nitrogen reserves of these soils is small. Other agencies which may be adding to the soils' nitrogen content are briefly considered.

INTRODUCTION

The three tussock-grassland soils examined in this series of investigations (Thornton 1958) contain considerable amounts of total nitrogen (0.27%–0.55% at a depth of 2–4 inches). The role of non-symbiotic bacteria in contributing to the soils' nitrogen content is of interest in view of the absence of indigenous legumes and hence of associated symbiotic nitrogen-fixing bacteria. Although the exotic clovers *Trifolium repens* and *T. dubium* occur at the three sites (Thornton 1958), their numbers are low and until their introduction to these sites within the last one hundred years they could not have added at all to the nitrogen content of the soils.

Recently, strains of the soil-inhabiting bacteria *Aerobacter* (Jensen 1956) and *Pseudomonas* (Anderson 1955) have been shown capable of fixing nitrogen, whilst Metcalfe and Chayen (1954) have recorded nitrogen fixation by yeasts, and Brown and Metcalfe (1957) by *Pullularia pullulans*. In this study, therefore, a search has been made both for *Azotobacter* and *Clostridium* and for other organisms which may be contributing to the maintenance of the soils' nitrogen content.

MATERIALS AND METHODS

Soils

A description of the Taupo soil near Waiouru, the Tekoa soil near Bealey, and the Omarama soil near Alexandra is given by Thornton (1958). Samples (about 20–50 g) were taken at a depth of 2–4 in.

N.Z. J. agric. Res. 1: 958–967.

during spring, 1955, 1956, and 1957, and summer, 1956 and 1957, from the base of *Festuca* tussock plants (tussock zone) and between well-spaced *Festuca* tussock plants (inter-tussock zone). Samples were collected in sterile screw-capped bottles and were normally used within 24 hours; otherwise, they were stored at 4°c.

Estimation of Nitrogen-fixing Clostridium spp.

Organisms of the *Clostridium butyricum* group were estimated in an *N-free medium (K_2HPO_4, 1 g; $MgSO_4.7H_2O$, 1 g; $FeCl_3.6H_2O$, 10 mg; $ZnSO_4.7H_2O$, 1 mg; $Na_2MoO_4.2H_2O$, 0.5 mg; glucose, 10 g; agar, 15 g; tap water, 1l) supplemented with an extract (10% v./v.) of Egmont brown loam (Ross 1958). Layers (10 ml) of this medium were allowed to set in petri dishes (10 cm diameter). Soil dilutions (1/20, 1/50, 1/100, 1/200) were prepared by shaking the soil (2–5 g, undried) vigorously by hand in sterile water for about 2 minutes. The soil dilution (1.0 ml) and molten medium (25 ml), at 45°c, were added to the previously set medium and mixed. The plates were incubated for 7 days at 24–28°c in the anaerobic jar of McIntosh and Fildes (Mackie and McCartney 1950) and a count then made of colonies staining blue-purple with aqueous iodine.

A typical colony of *Clostridium butyricum* was isolated from a dilution plate of the Taupo soil, and was purified by growing in a potato-glucose liquid medium, heating at 80°c for 5 minutes and subsequently sub-culturing anaerobically on potato glucose agar. The properties of this strain are identical with those given for *Clostridium butyricum* in Bergey's Manual (1948).

Estimation of Nitrogen-fixing Aerobic Bacteria

The following N-free basal medium was used for the isolation of aerobes: K_2HPO_4 1 g; $MgSO_4.7H_2O$, 1 g; $CaCO_3$, 2.5 g; $FeCl_3.6H_2O$, 10 mg; $ZnSO_4.7H_2O$, 1 mg; $Na_2MoO_4.2H_2O$, 0.5 mg; tap water, 1 l. The medium was supplemented with glucose (1%), or mannitol (1%), or sodium benzoate (0.1%). Media were solidified by the addition of agar (1.5%).

In Soil. Soil dilutions (1/10) were prepared by shaking as above and the suspensions (0.5 ml) spread over the surface of mannitol and benzoate agar plates (*cf.* Hussain Aleem 1953). Duplicate plates were usually used and were incubated at 24°c for 28 days.

In an enrichment technique, the soil (1g, undried) was added to liquid glucose medium (20 ml in 250 ml conical flasks) and incubated at 24°c for 28 days. A loopful of the resulting surface growth was spread over glucose agar plates and isolated colonies further sub-cultured and examined.

On Tussock Leaves. Leaves of *Festuca* tussock plants, collected at the site near Waiouru in spring, 1956, were chopped into small (about 5 mm) lengths and a few pieces spread over N-free glucose agar plates. In addition, an aqueous suspension of chopped leaves (about 1/50

* "N-free" media contain no added nitrogen compound.

w./v.) was shaken vigorously for 10 min. The supernatant liquid (0.5 ml) was spread over plates of the same medium and a further sample (5.0 ml) added to the N-free glucose liquid medium (20 ml in 250 ml conical flasks). After incubation at 24°C for 28 days, organisms from the enrichment cultures were isolated on glucose agar plates.

Nitrogen Fixation by Isolated Organisms

Aerobes were grown in the N-free glucose liquid medium (20 ml in 200 ml conical flasks) and also on plates of glucose agar (30 ml).

The N-free glucose liquid medium (10 ml in petri dishes, 10 cm diameter), supplemented with soil extract (0.5 ml) or raw potato sap (0.15 ml) (Ross 1958), was used for determining nitrogen fixation by the strain of *Clostridium butyricum*. Plates were inoculated with a suspension (0.05 ml) of the organism, previously grown for 24 hours in a potato glucose medium. These plates, together with uninoculated plates, were incubated in the same anaerobic jar at 24°C for 14 days. After incubation, the original inoculating suspension (0.05 ml), which had been stored at —20°C, was added to the uninoculated plates; these plates now served as the controls.

Nitrogen was determined by the Kjeldahl method. The digestion was made with sulphuric acid (50% v./v.) containing selenium dioxide (1% w./v.) and the ammonia formed distilled in the apparatus of Parnas and Wagner (Niederl and Niederl 1942). The distillate was collected and subsequently titrated in boric acid (1% solution in ethanol, 20% v./v.) containing the mixed bromocresol-green methyl-red indicator of Conway and O'Malley (1942).

Glucose was determined by the method of Somogyi (1952).

TABLE 1. COUNTS OF *Clostridium butyricum* IN SOILS
(Organisms per Gramme of Undried Soil)

Soil	Gas-producing Colonies		Total of Gas-producing and Non-aerogenic Colonies	
	Tussock Zone	Inter-tussock Zone	Tussock Zone	Inter-tussock Zone
Taupo soil (Waiouru)	1,170 (700–1,640)	1,250 (860–1,540)	9,000 (4,300–17,200)	5,840 (4,900– 8,000)
Tekoa soil (Bealey)	410 (150– 700)	230 (200– 250)	15,600 (10,500–22,700)	20,900 (16,400–25,000)
Omarama soil (Alexandra)	620 (300–1,380	1,230 (400–2,930)	19,700 (11,000–27,400)	20,000 (13,000–27,200)

1. Results are recorded as the means of four samples, collected from each zone in spring 1957, with the ranges given in parentheses.
2. The colonies counted were those producing a blue-purple colour with iodine solution.

RESULTS

Occurrence of Clostridium butyricum

In N-free glucose agar media, colonies of nitrogen-fixing clostridia are usually identified by morphology, by gas production, and by their characteristic staining reaction with iodine. In the present anaerobic plates, many colonies stained varying shades of brown and others blue-purple on the addition of aqueous iodine. The gas-producing, purple-staining colonies are typical of *Clostridium butyricum* (Swaby 1939) and counts of them are given in Table 1.

In view of the isolation by Tchan and Pochon (1950) of an anaerobe which did not give the blue stain with iodine and yet was reported to fix nitrogen, it is possible that some nitrogen-fixing anaerobes also occur amongst the present brown-staining colonies. However, unless each colony is examined separately for its nitrogen-fixing ability, there would appear to be no technique for discriminating between those capable and those incapable of fixing nitrogen. The brown-staining colonies usually differed morphologically from *Clostridium butyricum,* the strain of which examined here gave a blue, and not a brown stain, over an incubation period of 3–21 days. Counts of these brown-staining colonies were, therefore, not recorded in this study.

Morphologically, cells from non-aerogenic, blue-staining colonies, which ranged in size from 0.2–2.0 mm diameter, were similar to *Clostridium butyricum.* Although it is uncertain whether these bacteria are true nitrogen-fixers, they have been included in Table 1 for the following reasons: In the basal medium, with or without soil or potato extracts, an inoculum of *Clostridium butyricum*, which contained about 50% of spores, developed colonies which also ranged in size from 0.2–2.0 mm diameter and which frequently did not produce gas in the seven-day incubation period. Gas production must be partly related to the local availability of glucose and also to the stage of colony development which is, in turn, undoubtedly affected by the physiological state of the inoculum cells. In the soil, clostridia probably exist in both active and dormant states and their development in the isolation medium could be expected to differ considerably.

The numbers of *Clostridium butyricum* are similar in the three soils, as it is apparent from Table 1 that appreciable differences in counts occur between individual samples from the one sampling area. The ratio between gas-producing and total clostridial colonies varies considerably and could indicate a greater number of dormant forms in the Tekoa and Omarama soils. In all soils, no consistent difference is found between the tussock and inter-tussock zone samples.

The efficiency of nitrogen fixation by the present strain of *Clostridium butyricum* in the N-free glucose medium, supplemented with soil or potato extracts, was not particularly high with 3.7 (3.2–4.4) mg nitrogen fixed for each gramme of glucose utilised. This value is probably a little low, as Rosenblum and Wilson (1950) have shown that the addition of hydrogen to the atmosphere, as used in the present study in the anaerobic jar of McIntosh and Fildes, decreases the extent and efficiency although not the rate of fixation. Other strains in these soils may be more efficient at fixing nitrogen as Parker (1954) has shown that strain variation may be considerable.

Absence of Azotobacter *in Soils*

Equal numbers of samples from the tussock and inter-tussock zones of Taupo soil (20 samples), Tekoa soil (14 samples) and Omarama soil (14 samples), on examination by the plate dilution method on mannitol and benzoate agar, showed no colonies typical of *Azotobacter* spp. When a few cells of authentic *Azotobacter chroococcum* were added to the suspensions of inter-tussock zone samples of Tekoa and Omarama soils, characteristic colonies of the organism readily developed.

As the above method would not regularly detect less than ten *Azotobacter* cells per gramme of soil, a study by the enrichment technique was made of sixty-six samples, comprising those listed above with further samples from both zones of the Taupo soil. All samples gave extensive surface growth which consisted chiefly of fungal hyphae but included many bacteria. Sub-culture on the N-free glucose agar, however, showed *Azotobacter* spp. to be absent. Proof that the native microflora of the soil was not inhibitory to the development of *Azotobacter* was shown by the ready isolation by this technique of *A. chroococcum, A. agilis, A. vinelandii*, and *A. beijerinckii* which had been added (20 to 2,000 organisms per flask) to cultures of the Taupo soil.

Nitrogen-fixing Properties of Other Soil Bacteria

From all soils, bacteria were isolated by the enrichment technique but those that failed to survive sub-culturing twice on the N-free glucose agar were discarded. From thirty samples of Taupo soil, the remaining cultures, consisting chiefly of small Gram-negative rods, could be divided into two groups.

Eleven strains of the larger group were examined. In their growth in veal broth, in litmus milk, and on potato slopes, in their utilisation of glucose, sucrose, mannitol, starch, and nitrate, and in their production of hydrogen sulphide, the properties of this group were identical with those of *Bacterium radiobacter* (Hofer 1941). On mannitol glycerophosphate agar, however, the cream-coloured mucoid growth did not possess the brown halo said to be characteristic of this organism. One strain liquefied gelatin but the others did not. Conn and Elrod (1947) state that flagellation is variable in the *Agrobacterium* group among which *Bacterium radiobacter* is now classified (Bergey's Manual, 1948). The two strains here examined possessed one to three sub-polar flagella. The isolates were distinguished from rhizobia associated with clovers by their failure to produce nodules on inoculation of white clover, *Trifolium repens,* according to the method of Purchase and Vincent (1949).

In the second group, three strains, two of which were pigmented yellow-green or brown on different media, were selected on the basis of their biochemical properties as being members of the *Pseudomonas* group.

Growth of both these groups of bacteria in the N-free glucose liquid medium was poor, but was similar to that described by Anderson (1955) for a nitrogen-fixing *Pseudomonas.* The calcium carbonate particles were bound together with gum formed by the organisms whilst the culture fluids remained practically clear. The nitrogen contents of these cultures after growth of the organisms for 28 days are recorded in Table 2, and it is apparent that nitrogen fixation, if occurring at all, is insignificant. Jensen (1956) found that the incorporation of a little agar or yeast extract in an N-free glucose medium greatly stimulated fixation of nitrogen by two strains of *Aerobacter aerogenes.* The present cultures were grown on both these media but the growth was again very poor after 28 days and visually no greater than before.

TABLE 2. Glucose Utilisation and Nitrogen Content of N-free Media after Growth of Some Soil Bacteria

Medium	Bacterial Group	No. of Strains Studied	Glucose Utilised mg/ml	Nitrogen Content of Medium after Growth, μg/ml
liquid	none	—	—	4.2
liquid	*Agrobacterium radiobacter*	11	2.3 ± 0.3 (0.7–4.7)	4.5 ± 0.2 (4.0–5.9)
liquid	*Pseudomonas* spp.	3	0.3 ± 0.2 (0–0.5)	4.2 ± 0.2 (4.0–4.4)
solid	none	—	—	10.4
solid	*Agrobacterium radiobacter*	4	—	10.9 ± 0.9 (8.8–12.3)
solid	*Pseudomonas* spp.	1	—	9.3

1. Crltures were incubaten for 28 days in the N-free liquid medium and 14 days on N-free glucose agar. After this growth period, uninoculated liquid medium contained 8.9 mg glucose/ml.
2. Results are recorded as means ± standard error, the ranges being given in parentheses.

In contrast, members of the *Agrobacterium radiobacter* group grew well on N-free glucose agar producing, after 7 days, colourless, transparent, gummy colonies (0.5–4 mm diameter). The three *Pseudomonas* spp. produced colourless, translucent colonies (0.5–1.5 mm diameter) on this medium but growth was not vigorous. The most strongly growing strains of each group, however, failed to fix any appreciable quantity of nitrogen on this medium (Table 2).

Bacteria from the enrichment cultures of the Tekoa and Omarama soils were not studied in detail but appeared to resemble those from the Taupo soil. After growth of nine of these organisms on N-free glucose agar for 28 days, the nitrogen content of the medium was

0.127 ± 0.004 mg, range 0.108–0.144 mg, the nitrogen content of uninoculated controls being 0.129 mg.

Organisms from Leaves of Festuca *Tussock Plants*

When leaves of *Festuca* tussock plants were plated directly on N-free glucose agar, very extensive mould growth occurred and no colonies of *Azotobacter* were seen. When six different aqueous suspensions were plated on this medium, however, many bacteria-like colonies were obtained and were sub-cultered twice on the N-free glucose agar. Examination of typical colonies (14), greater than 0.5 mm diameter after incubation for 7 days, showed the presence of *Bacillus subtilis, B. firmus* and *B. lentus* (2) (identified by the methods of Smith *et al.* 1952), *Pseudomonas* spp. (2), the yeast *Cryptococcus laurentii* (4), and *Pullularia pullulans* (4). The enrichment method yielded the yeast *Rhodotorula mucilaginosa*, *Serratia* sp., and many unidentified rods and cocco-bacilli producing watery, mucoid colonies. No *Azotobacter* spp. were seen by any isolation technique.

None of the isolated organisms showed visible growth in the N-free glucose liquid medium. After growth of *Cryptococcus laurentii, Pullularia pullulans,* and the *Bacillus* spp. for 14 days on the solidified medium, the mean nitrogen content was 0.162 ± 0.012 mg, range 0.133–0.180 (*Pullularia pullulans,* 0.150 mg), the mean nitrogen content of uninoculated controls being 0.156 mg.

DISCUSSION

In many natural-grassland areas of the world, indigenous legumes are absent (Whyte *et al.* 1953) and the origin of the soils' nitrogen content is uncertain. Parker (1957) has presented evidence of nitrogen accumulation in grassland soils and attributes an important role to the activity of free-living bacteria. From the results of the present study, however, it would appear that, at the present time, non-symbiotic bacteria are contributing only a little to the nitrogen content of these tussock-grassland soils.

The presence of nitrogen-fixing clostridia in these soils confirms their wide distribution in temperate countries, for other workers, such as Swaby (1939) and Jensen (1940) in Australia, have found them in all soils examined. The numbers of gas-producing colonies are comparable with those found by Swaby in Victorian soils although the numbers reported for these Australian soils may be somewhat low owing to the use of unenriched medium (Ross 1958). Much higher numbers have been reported for an American soil by Willis (1934) who counted all organisms appearing on N-free glucose agar plates. However, it is well known that many aerobes incapable of nitrogen fixation can grow on medium without added nitrogen and it would, therefore, appear unwise to accept as nitrogen-fixers all bacteria that can grow in these media under anerobic conditions. The present method used for enumerating *Clostridium butyricum* does not distinguish between spores and vegetative forms.

At none of the sites were members of the *Azotobacter* group detected. The absence of *A. chroococcum* in the Taupo and Tekoa soils is probably associated with the acidity of these soils, as a pH below 6.0 does not normally favour growth of this species (Jensen 1955). The non-detection of the more acid-tolerant species, viz. *A. beijerinckii* and *A. macrocytogenes* (Jensen 1955) would indicate that these organisms are not widely distributed. In his extensive survey of Australian wheat soils, Jensen (1940) found that *A. chroococcum* was present in only half of the soils above pH 6.0 and that *A. beijerinckii* was seldom detected.

Although growing well on media to which no nitrogen has been added, members of the *Agrobacterium radiobacter* group and the other soil bacteria here studied cannot be regarded as contributing appreciably to nitrogen accumulation in these soils. At the most, nitrogen fixation by these organisms in pure-culture is very low. The *Agrobacterium radiobacter* group has been classified by many workers among the nitrogen-fixing bacteria, but Lindstrom *et al.* (1951) have shown by the use of ^{15}N that the strains examined by them fixed, if at all, only very small amounts of nitrogen.

No evidence has been obtained of nitrogen fixation by bacteria and yeasts from the leaves of *Festuca* tussock plants. Di Menna (1958) has found that many yeasts isolated from these tussock-grassland soils and from the leaves of tussock plants can grow in media to which only trace amounts of nitrogen have been added, but in no instance was there a vigorous growth indicative of strong nitrogen fixation. Brown and Metcalfe (1957) have shown *Pullularia pullulans*, isolated from soil, to be capable of nitrogen fixation, but a strain isolated in this work does not possess comparable nitrogen-fixing properties.

Owing to the low number of nitrogen-fixing bacteria in these soils, it is of value to consider some of the other agencies which may have contributed to the soils' nitrogen content. Blue-green algae have been found in the three soils by Flint (1958), but only in the Omarama soil near Alexandra would conditions be expected to favour their development. Their present contribution to the nitrogen content of the soils is probably not large.

Under tropical conditions, Ruinen (1956) has found *Beijerinckia* on the leaves of many plants and has postulated an important role for them in the plants nitrogen economy. A similar association between nitrogen-fixing bacteria and leaves has not been shown for plants in temperate countries although Stevenson (1953) considered the possibility of some plants being independent of combined nitrogen through the absorption of atmospheric nitrogen by leaf and stem structures.

A few genera of Angiosperms, other than legumes, have been shown capable of nitrogen fixation through the possession of root nodules inhabited by symbiotic micro-organisms (Bond and Gardner 1957). The possibility that similar plants have contributed to the nitrogen content of these tussock-grassland soils is an interesting one but there is no evidence of their occurrence at the present sites. Druce (1957) has noted that all the species of *Coriaria* in New Zealand are vigorous colonisers of sands and gravels and that some, at least, stimulate the growth of associated species. It would appear probable that if plants such as *Coriaria*, which possess root nodules, and are stated by MacConnell and Bond (1957) to have associated symbiotic micro-organisms, have contributed to the nitrogen content of these low tussock grassland soils they would have done so as pioneer plants and not as permanent members of the grassland complex (Mr. A .P. Druce, pers. comm.).

ACKNOWLEDGEMENTS

I wish to thank Dr R. H. Thornton for providing soil and leaf samples, Dr Y. T. Tchan, University of Sydney, for cultures of *Azotobacter*, and Mr W. S. Bertaud, Dominion Physical Laboratory, Lower Hutt, New Zealand, for the electron micrographs of flagella. Yeasts and *Pullularia pullulans* were kindly identified by Dr M. E. di Menna.

REFERENCES

ANDERSON, G. R. 1955: Nitrogen Fixation by *Pseudomonas*-like Soil Bacteria. *J. Bact. 70:* 129-133.

"BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY." Edited by Breed, R. S.; Murray, E. G. D.; Hitchens, A. P. 1948: 6th ed. Williams and Wilkins, Baltimore.

BOND, G.; GARDNER, I. C. 1957: Nitrogen Fixation in Non-legume Root Nodule Plants. *Nature, Lond. 179:* 680-1.

BROWN, M. E.; METCALFE, G. 1957: Nitrogen Fixation by a Species of *Pullularia. Nature, Lond. 180:* 282.

CONN, H. J.; ELROD, R. P. 1947: Concerning Flagellation and Motility. *J. Bact. 54:* 681-7.

CONWAY, E. J.; O'MALLEY, E. 1942: Microdiffusion Methods. Ammonia and Urea Using Buffered Absorbents (Revised Methods for Ranges Greater than 10 μg N). *Biochem. J. 36:* 655-61.

DRUCE, A. P. 1957: Botanical Survey of an Experimental Catchment, Taita, New Zealand. *N.Z. Dep. sci. industr. Res. Bull. 124.*

FLINT, E. 1958: Biological Studies of Some Tussock Grassland Soils. IX. Algae. Preliminary Observations. *N.Z. J. agric. Res. 1:* 991-7.

HOFER, A. W. 1941: A Characterisation of *Bacterium radiobacter* (Beijerinck and van Delden)Löhnis. *J. Bact. 41:* 193-224.

HUSSAIN ALEEM, I. M. 1953: Counting of *Azotobacter* in Soils. *Plant & Soil 4:* 248-51.

JENSEN, H. L. 1940: Contributions to the Nitrogen Economy of Australian Wheat Soils, with Particular Reference to New South Wales. *Proc. Linn. Soc. N.S.W. 65:* 1-122.

——1955: *Azotobacter macrocytogenes* n. sp., a Nitrogen-fixing Bacterium Resistant to Acid Reaction. *Acta. agric. Scand. 5:* 278-294.

JENSEN, V. 1956: Nitrogen Fixation by Strains of *Aerobacter aerogenes. Physiol. Plant. 9:* 130-6.

LINDSTROM, E. S.; LEWIS, S. M.; PINSKY, M. J. 1951: Nitrogen Fixation and Hydrogenase in Various Bacterial Species. *J. Bact. 61:* 481-7.

MACCONNELL, J. T.; BOND, G. 1957: A Comparison of the Effect of Combined Nitrogen on Nodulation in Non-legumes and Legumes. *Plant & Soil 8:* 378-88.

MACKIE, T. J.; MCCARTNEY, J. E. 1950: "Handbook of Practical Bacteriology." 8th ed. Livingstone, Edinburgh.

MENNA, M. E. DI 1958: Some Physiological Studies of Yeasts from Soils and Allied Habitats. *J. gen. Microbiol.* (In the Press).

METCALFE, G.; CHAYEN, S. 1954: Nitrogen Fixation by Soil Yeasts. *Nature, Lond. 174:* 841.

NIEDERL, J. B.; NIEDERL, V. 1942: "Micromethods of Quantitative Organic Analysis." 2nd ed. John Wiley, New York.

PARKER, C. A. 1954: Non-symbiotic Nitrogen-fixing Bacteria in Soil. I. Studies on *Clostridium butyricum*. *Aust. J. agric. Res. 5:* 90-7.

————1957: Non-symbiotic Nitrogen-fixing Bacteria in Soil. III. Total Nitrogen Changes in a Field Soil. *J. Soil. Sci. 8:* 48-59.

PURCHASE, H. F.; VINCENT, J. M. 1949: A Detailed Study of the Field Distribution of Strains of Clover Nodule Bacteria. *Proc. Linn. Soc. N.S.W. 74:* 227-36.

ROSENBLUM, E. D.; WILSON, P. W. 1950: Molecular Hydrogen and Nitrogen Fixation by *Clostridium*. *J. Bact. 59:* 83-91.

ROSS, D. J. 1958: Influence of Media on the Counts of *Clostridium butyricum* in Soils. *Nature, Lond. 181:* 1172-3.

RUINEN, J. 1956: Occurrence of *Beijerinckia* species in the "Phyllosphere". *Nature, Lond. 177:* 220-1.

SMITH, N. R.; GORDON, R. E.; CLARK, F. E. 1952: Aerobic Spore-forming Bacteria. *U.S. Dep. Agric., agric. Monogr. 16.*

SOMOGYI, M. 1952: Notes of Sugar Determination. *J. biol. Chem. 195:* 19-23.

STEVENSON, G. B. 1953: Bacterial Symbiosis in Some New Zealand Plants. *Ann. Bot. n. s. 17:* 343-6.

SWABY, R. J. 1939: The Occurrence and Activities of *Azotobacter* and *Clostridium butyricum* in Victorian Soils. *Aust. J. exp. Biol. med. Sci. 17:* 401-423.

TCHAN, Y. T.; POCHON, J. 1950: Une Espèce Nouvelle de Bactérie Fixatrice d' Azote Moléculaire Isolée du Sol: *Endosporus azotophagus* n. sp. *C.R. Acad. Sci., Paris 230:* 417-8.

THORNTON, R. H. 1958: Biological Studies of Some Tussock-grassland Soils. I. Introduction, Soils and Vegetation. *N.Z. J. agric. Res. 1:* 913-21.

WHYTE, R. O.; NILSSON-LEISSNER, G.; TRUMBLE, H. C. 1953: Legumes in Agriculture. *FAO Agric. Stud. 21.*

WILLIS, W. H. 1934: The Metabolism of Some Nitrogen-Fixing *Clostridia. Iowa agric. Exp. Sta. Res. Bull. 173.*

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

VI. NITRIFYING ACTIVITIES

By D. J. Ross, Soil Bureau, Department of Scientific and Industrial Research, Wellington.

(Received for publication, 19 June 1958)

Summary

Nitrifying organisms occur in the soils of three New Zealand tussock grasslands. A low nitrifying potential, particularly for the oxidation of nitrite, is shown by the moderately to strongly acid Tekoa soil near Bealey. The Taupo soil near Waiouru and the Omarama soil near Alexandra show much greater activity. An increase in activity has not been found at the base of *Festuca* tussock plants. The effect of seasons upon nitrifying activity has been briefly studied.

INTRODUCTION

The soils of three New Zealand tussock grasslands contain considerable total nitrogen (Thornton 1958). Its rate of mineralisation and the role of micro-organisms in this process have not previously been examined. In this series of investigations, bacteria capable of proteolysis and ammonification have been found by Stout (1958), although at none of the sites were strong ammonifiers isolated. It would, therefore, appear probable that bacteria contribute only slowly to the ammonia content of these soils. In the present study, organisms oxidising ammonia to nitrite and nitrate are investigated by measuring the nitrifying activities of the soils *in vitro*.

MATERIALS AND METHODS

A description of the Taupo soil near Waiouru, the Tekoa soil near Bealey, and the Omarama soil near Alexandra is given by Thornton (1958). These three tussock-grassland soils are considered to be of low natural fertility. Mineral deficiencies are mainly responsible for the low fertility of the Taupo and Tekoa soils, whereas lack of moisture is a major factor limiting fertility of the Omarama soil. Soils were sampled as before (Ross 1958) at a depth of 2–4 in. at the base of *Festuca* tussock plants (tussock zone) and between well-spaced *Festuca* tussock plants (inter-tussock zone). Samples of the Taupo soil were taken in summer (February 1955), winter (August 1955), and in spring (October 1955), and samples of Tekoa and Omarama soils were collected in spring (November 1956).

Although the numbers of samples studied are too small for detailed analysis, they illustrate major features associated with the nitrifying activities of these soils.

Media and Cultural Conditions

The media were those used by Mulder (1950) and are of the following composition:

N.Z. J. agric. Res. 1; 968–973.

Medium A for Nitrite Producers: K_2HPO_4, 0.5 g: $MgSO_4.7H_2O$, 0.5 g; $Fe(NH_4)_2(SO_4)_2.6H_2O$, 0.25 g; $CaCO_3$, 10 g; tap water, 950 ml. The medium (19 ml in 100-ml conical flasks) was sterilised at 15 lb/in.^{2} for 15 min, and $(NH_4)_2SO_4$ solution (2 %, 1.0 ml) then added after separate sterilisation.

Medium B for Nitrate Producers: K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.3 g; Na_2CO_3, 1.0 g; NaCl, 0.5 g; $FeSO_4.7H_2O$, 0.4 g; tap water, 950 ml The medium was distributed and sterilised as above and sterile $NaNO_2$ solution (2%, 1.0 ml) then added.

The undried soil (1.0 g) was added to the media, and the flasks were incubated in the dark, without shaking, at 24°c. It is realised that, under these cultural conditions, the activity of the nitrifying organisms may be handicapped by slowness in diffusion of oxygen through the medium. However, it is clear from the experimental results that very wide differences in nitrifying activity are found between different soils and that a possible deficiency in the oxygen content of the culture medium is not the main factor limiting nitrifying activity in these experiments.

Chemical Estimations

The culture fluids were examined at weekly intervals but, for simplicity in presentation, results are recorded only for the incubation periods indicated in Tables 1 and 2. The measurement of the activity of organisms oxidising ammonia was made by estimating residual ammonia in medium A and that of organisms oxidising nitrite by measuring residual nitrite in medium B.

Ammonia was determined with Nessler's reagent, the colour produced being measured with the "Spekker" photoelectric absorptiometer using Kodak no. 1 blue filters. Although this method may be subject to interference from factors such as other nitrogen compounds in the soil, it is considered sufficiently accurate for the purpose of this study. It has been established for all cultures that the disappearance of ammonia is always associated with the appearance of nitrite. As this nitrite, however, is usually further metabolised, its accumulation is not normally recorded in this work.

Nitrate was estimated with Griess-Illosvay's reagent, the colour produced being measured with the "Spekker" using Kodak no. 4 green filters. Under the present cultural conditions with shallow layers of medium and no added organic compounds to act as energy sources, the activity of denitrifying organisms would not be favoured and it can reasonably be deduced that the utilisation of nitrite results from its oxidation by nitrifying organisms. In those cultures from which nitrite had completely disappeared, tests for nitrate were made and always found positive by the method of Zobell (1932).

In Tables 1 and 2, the amounts of ammonia and nitrite oxidised are expressed as a percentage of the amounts originally present in the culture media. Because of wide variations in the activities of samples from the same site, the individual activities of all samples are recorded in these tables.

RESULTS

Influence of Festuca *Tussock Plants*

It is clear from Table 1 that the roots of growing *Festuca* tussock plants do not encourage an increase of nitrifying organisms, for no marked difference is noted between the nitrifying activities of samples from the tussock and inter-tussock zones.

TABLE 1. INFLUENCE OF *Festuca* TUSSOCK PLANTS AND SOIL ON NITRIFYING ACTIVITY

Soil	Percentage Oxidised			
	Ammonia		Nitrite	
	Tussock Zone	Inter-tussock Zone	Tussock Zone	Inter-tussock Zone
Taupo soil (Waiouru)	0 7 32 61 (25)	47 69 73 88 (69)	0 10 99 99 (52)	4 16 71 100 (48)
Tekoa soil (Bealey)	0 0 21 37 (15)	0 3 10 21 (9)	0 0 0 0 (0)	0 0 0 5 (1)
Omarama soil (Alexandra)	14 49 51 54 (42)	13 48 51 53 (41)	20 38 100 100 (65)	59 100 100 100 (90)

Four 1-g samples from each zone were incubated for 35 days in culture media (20 ml) containing 4.6 mg NH_4^+—N or 4.1 mg NO_2^-—N per flask. Means of the results are given in parentheses.

Influence of Soil

The nitrifying activity of a soil depends considerably on its other properties. The activities of the Tekoa soil samples are low, whilst samples of the Omarama soil show the greatest activity. Even after incubation for 49 days, only 34%, range 13%–67%, of the original concentration of ammonia has been oxidised in medium A by the samples of Tekoa soil and only a very slight increase in the oxidation of nitrite, over that reported in Table 1, was apparent.

From the results of Table 1, it can be inferred that, in all soils, the distribution of nitrifying organisms varies considerably. The ammonia content of medium A was not lowered by one sample of

Taupo soil within the incubation period of 35 days. Half of the samples of Taupo soil either failed to oxidise nitrite, or did so only to a slight extent, although the other samples of Taupo soil showed strong activity. In the 35-day incubation period, half of the samples of Tekoa soil from each zone produced only traces of nitrite and either a slight, or non-detectable, lowering of the ammonia content of medium A. Slight oxidation of nitrite was shown by only one sample of Tekoa soil from the inter-tussock zone. Even on incubation for 49 days, six of the eight samples studied failed to oxidise nitrite. Over the 49-day incubation period, these six samples of Tekoa soil produced nitrite (corresponding to 32%, range 14%–43% of the original concentration of ammonia) in medium A, but there was no evidence, either in this medium or in the nitrite medium B, of the further oxidation of nitrite to nitrate. Within the incubation period of 35 days, all samples of Omarama soil oxidised ammonia and most of them oxidised nitrite strongly.

Influence of Season

With the Taupo soil, the greatest activity has been shown by summer samples (Table 2). Differences from the spring samples are, however, not appreciable and there is again considerable variation between the individual samples of each collection. A sample of the Tekoa soil from the inter-tussock zone, collected in February 1955, showed no activity during an incubation period of 35 days. Seasonal changes would be expected to be very slight in the Tekoa soil because of the generally low level of nitrifying activity in this area.

TABLE 2. Influence of Season on Nitrifying Activity of Taupo Soil (Waiouru)

Percentage Oxidised					
Ammonia			Nitrite		
Spring	Summer	Winter	Spring	Summer	Winter
0	37	8	0	0	0
6	65	13	0	99	15
13	(51)	14	2	(50)	15
29		30	3		17
36		(16)	20		(12)
44			77		
46			98		
50			98		
(28)			(37)		

Samples (Spring, 8; summer, 2; winter, 4) were taken in equal numbers from the tussock and inter-tussock zones and the results from the two zones combined. Cultural conditions were the same as for Table 1, but an incubation period of 28 days was employed. Means of the results are given in parentheses.

Discussion

As the autotrophic *Nitrosomonas* and *Nitrobacter* play the major role in the nitrification process, it would not be expected that the excretion or degradation products of plant roots would assist in this process except for the possible provision of ammonia, resulting from the metabolic activities of rhizosphere micro-organisms, and the encouragement of heterotrophic nitrite producers known to be present in soils (Fisher *et al.* 1956). *Festuca* tussock plants are here shown not to stimulate nitrification directly, the results thus agreeing with those of Goring and Clark (1948) who found no difference between the numbers of nitrifying organisms from either grass-root soils or fallow soils although, in general, microbial numbers were higher around plant roots.

The large differences found between the nitrifying activities of different samples from each of the three soils could reflect the irregular distribution of nitrifying organisms in these tussock-grassland soils. Judged by the low rates of nitrification of all samples taken, these organisms appear to be present in only very small numbers in the Tekoa soil. The samples of Tekoa soil show, in particular, a very much slower oxidation of nitrite than of ammonia and so differ from most soils which oxidise nitrite more rapidly than ammonia (Russell 1953).

In the Taupo and Tekoa soils, the contents of "citric-soluble" phosphate and exchangeable calcium fall within the medium-range group for New Zealand soils (Thornton 1958), whilst in the Omarama soil the contents of both components are high. The nitrifying activities of these soils must be influenced in part by the concentrations of these elements, which are known to favour nitrification (Meiklejohn 1954), although the correlation between phosphorus content and nitrifying activity is not particularly marked. A closer correlation is found between the rates of nitrification and the acidity of the soils with the more acid soils giving slower nitrification, as found, for example, by Richardson (1938) in other studies.

It is hoped, in a further study, to investigate the nitrifying activities of more-fertile agricultural soils in New Zealand and to compare them with those of some tussock-grasslands soils both before and after agricultural development. Present results would indicate that the Taupo and Omarama soils are potentially capable of moderate nitrifying activity. In the Tekoa soil, however, the rates of both stages in the nitrification process are so slow that they would probably result in a shortage, in the field, of nitrate available for plant growth. In pot experiments, Morrison (1958) has found that the addition of ammonium nitrate to the Tekoa soil, previously enriched with phosphate, results in a considerable increase in the growth of *Festuca* tussock plants.

ACKNOWLEDGEMENTS

I wish to thank Dr. R. H. Thornton for collecting soil samples.

REFERENCES

FISHER, T.; FISHER, E.; APPLEMAN, M. D. 1956: Nitrite Production by Heterotrophic Bacteria. *J. gen. Microbiol. 14:* 238-47.

GORING, C. A. I.; CLARK, F. E. 1948: Influence of Crop Growth on Mineralization of Nitrogen in the Soil. *Proc. Soil Sci. Soc. Amer. 13:* 261-6.

Meiklejohn, J. 1954: Some Aspects of the Physiology of the Nitrifying Bacteria. *In* "Autotrophic Micro-organisms", *4th Symp. Soc. gen. Microbiol.* pp. 68-83.

Morrison, T. M. 1958: Biological Studies of Some Tussock-grassland Soils. XI. The Response of a Tussock Plant, *Festuca novae-zelandiae,* to Fertilisers. *N.Z. J. agric. Res. 1:* 1003-5.

Mulder, E. G. 1950: Effect of Liming of an Acid Peat Soil on Microbial Activity. *Trans. 4th int. Congr. Soil Sci. 1:* 117-21.

Richardson, H. L. 1938: The Nitrogen Cycle in Grassland Soils: With Especial Reference to the Rothamsted Park Grass Experiment. *J. agric. Sci. 28:* 73-121.

Ross, D. J. 1958: Biological Studies of Some Tussock-grassland Soils. V. Non-symbiotic Nitrogen-fixing Bacteria. *N.Z. J. agric. Res. 1:* 958-67.

Russell, E. W. 1953: "Soil Conditions and Plant Growth." 8th ed. p. 291 Longmans, Green, London.

Stout, J. D. 1958: Biological Studies of Some Tussock-grassland Soils. IV. Bacteria. *N.Z. J. agric. Res. 1:* 943-57.

Thornton, R. H. 1958: Biological Studies of Some Tussock-grassland Soils. I. Introduction, Soils and Vegetation. *N.Z. J. agric. Res. 1:* 913-21.

Zobell, C. E. 1932: Factors Influencing the Reduction of Nitrates and Nitrites in Semisolid Media. *J. Bact. 24:* 273-81.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

VII. PROTOZOA

By J. D. STOUT, Soil Bureau, Department of Scientific and Industrial Research, Wellington.

(*Received for publication, 19 June 1958*)

Summary

The microfauna occurring in cultures of three tussock-grassland soils has been examined through three seasons.

All three soils had a typical grassland fauna. Of the two major protozoan groups studied, the rhizopods and the ciliates, the ciliate fauna was in each case larger than the rhizopod fauna and the populations also appeared to be higher. The rhizopod fauna was similar in the three soils, but a number of ciliate species were confined to the soil with a high base status. The average incidence of the protozoan species was characteristic for each soil type and was slightly higher in the tussock zone than in the inter-tussock zone. The distribution of the rest of the fauna was generally similar in the three soils.

Neither moisture nor food appeared to be limiting factors and the most favourable ecological conditions obtained in the soil with the lowest rainfall and the poorest plant cover.

INTRODUCTION

The present paper gives an account of the microfauna of three tussock-grassland soils described in detail elsewhere (Thornton 1958). The three soils were derived from different parent materials, developed under different climates, and have had different vegetational histories. Consequently they show differences in weathering and leaching and in their organic fraction. The three soils are: the Omarama soil near Alexandra, a fine sandy loam high in exchangeable bases and with a very high base saturation with reduced and senile tussock cover; the Tekoa soil near Bealey, a silt loam of medium base status formerly covered by beech forest but now under tussock and introduced grasses; and the Taupo soil near Waiouru, a sandy silt of medium base status and with a vigorous plant cover of tussock and other grasses.

No attempt has been made to count directly the number of protozoa in the soils, and the relative populations have been estimated on the basis of the number of species occurring in a culture of 10 g of soil. This has been found to be a quite satisfactory basis for comparing the fauna of the different soil types in New Zealand and is believed to give a reliable comparative picture of the soil fauna. Such figures compare favourably with the total number of species identified in a particular soil and generally with the distribution of non-protozoan fauna. This approach, however, gives far greater prominence to the ciliates than to the rhizopods which reverses the prevailing view upon their relative importance based on population estimates (Meiklejohn 1955, p. 176). These counting techniques rely on growth following the

N.Z. J. agric. Res. 1: 974–984.

addition of concentrated suspensions of bacteria (Singh 1955) although this is a notoriously selective procedure and does not simulate a soil culture or environmental conditions which obtain in nature (Stout 1956a). For this reason it is considered that the non-enrichment culture method employed in this study is a more valuable means of evaluating the ecology of the protozoan and non-protozoan faunas.

MATERIALS AND METHODS

Soil samples were collected in 4-oz screw-capped jars. Samples were taken from the topsoil (2-4 in.) between tussock plants (inter-tussock samples) and the topsoil near to the tussock plants (tussock samples) and also at a depth of 12–14 in. (subsoil samples). Cultures were set up as soon as possible after the arrival of the sample at the laboratory. Petri dishes (10 cm) were used and the bottom covered with 2% plain agar: 10 g of soil was added to one side of the dish, and then 20 ml of sterile distilled water. The culture was examined with low power and water-immersion lenses. Cultures were examined two or three times a week for the first month of culture and were discarded after six months.

TABLE 1. AVERAGE NUMBER OF PROTOZOAN SPECIES IN THE SOIL CULTURES FOR THE THREE SEASONS AND THE THREE ZONES

T—tussock zone; I—inter-tussock zone; S—subsoil zone.

Rhizopods

	Taupo Soil (Waiouru)			Tekoa Soil (Bealey)			Omarama Soil (Alexandra)		
	T	I	S	T	I	S	T	I	S
Spring	7	8	3	4	5		7	7	
Summer	4	4		9	7		10	8	
Autumn	8	8	1	8	9	2	8	7	4
Total average	6.4	6.6		7.2	6.9		8.0	7.6	

Ciliates

	Taupo Soil (Waiouru)			Tekoa Soil (Bealey)			Omarama Soil (Alexandra)		
	T	I	S	T	I	S	T	I	S
Spring	9	7	2	12	11		18	14	
Summer	8	8		12	10		19	15	
Autumn	11	8	4	13	12	6	21	17	6
Total average	9.4	7.4		12.0	11.1		19.3	15.4	

Attention was concentrated on the topsoil samples where the protozoa are most active. A series of subsoil samples from Waiouru were also studied as well as samples from the other two areas. Typically a single culture was set up from each of three samples from each of the two zones (tussock and inter-tussock). In the summer Waiouru series, however, duplicate cultures were set up from each sample.

Results

Quantitative

Table 1 gives the average number of species in each series of cultures for each soil, each zone, and each season. Tables 2 and 3

TABLE 2. Total Incidence of Rhizopod Species, Listed Systematically, in the Three Soils During the Three Seasons in the Topsoil Cultures I—spring; II—Summer; III—Autumn.

Species	Taupo Soil (Waiouru)			Tekoa Soil (Bealey)			Omarama Soil (Alexandra)		
	WI	WII	WIII	BI	BII	BIII	AI	AII	AIII
Biomyxa vagans	3	2	2	—	4	5	—	3	3
Nuclearia simplex	5	—	6	—	2	5	3	—	3
Mayorella vespertilio	5	6	4	5	6	5	4	6	6
Trichamoeba sp.	6	3	5	3	5	6	4	6	4
Trichamoeba sp.	4	—	2	—	—	—	—	1	2
Thecamoeba striata	—	1	—	—	1	—	—	—	—
Thecamoeba verrucosa	—	—	—	—	—	—	1	2	—
Cryptodifflugia oviformis	—	—	1	—	—	—	2	6	4
Difflugia constricta	3	1	6	5	6	5	6	6	6
Difflugia arcula	6	4	5	3	1	2	2	—	—
Difflugia globulus	3	1	3	1	4	5	3	4	3
Difflugia lucida	—	—	—	—	—	—	—	3	2
Arcella sp.	—	—	—	—	—	—	—	—	2
Heleopora sp.	—	—	—	—	—	—	—	1	—
Trinema lineare	5	4	6	6	6	6	4	6	5
Trinema enchelys	4	—	2	1	1	4	4	6	2
Euglypha rotunda	2	2	5	1	5	5	4	2	2
Euglypha ciliata	—	—	—	1	—	—	3	—	1
Euglypha cristata	—	—	—	1	—	—	—	—	—
Euglypha denticulata	—	—	1	—	1	—	—	—	—
Sphenoderia dentata	—	—	—	1	6	1	1	1	—
Raphidiophrys elegans	—	—	—	—	—	2	1	1	—
Total number of species (TS)	11	9	13	11	13	12	14	15	14
Total species—incidence (TI)	46	24	48	28	48	51	42	54	45
Average species—incidence (TI/TS)	4.2	2.6	3.7	2.5	3.7	4.2	3.0	3.6	3.2

TABLE 3. TOTAL INCIDENCE OF CILIATE SPECIES, LISTED SYSTEMATICALLY, IN THE THREE SOILS DURING THE THREE SEASONS IN THE TOPSOIL CULTURES I—spring; II—summer; III—autumn.

Ecological Groups	Species	Taupo Soil (Waiouru)			Tekoa Soil (Bealey)			Omarama Soil (Alexandra)		
		WI	WII	WIII	BI	BII	BIII	AI	AII	AIII
a	*Enchelys* sp.	6	6	6	—	6	6	1	6	4
b	*Spathidium* sp.	—	1	1	3	1	3	4	5	6
b	*Chaenea* sp.	2	1	2	2	1	—	—	4	—
b	*Lacrymaria* sp.	—	2	—	1	1	4	—	2	2
b	*Lacrymaria olor*	2	—	—	1	—	—	—	—	—
b	*Trachellophyllum* sp.	—	—	—	—	—	1	1	—	1
b	*Prorodon* sp.	—	—	—	1	—	—	3	—	—
b	*Gastronauta membranaceus*	—	—	—	—	—	—	—	—	1
b	*Chilodonella gourandi*	—	—	—	—	—	—	1	—	—
b	*Chilodonella bavariensis*	—	—	—	1	—	—	1	—	1
b	*Chilodonella* sp.	2	1	2	—	—	—	—	1	1
b	*Lionotus* sp.	1	—	3	—	—	1	—	—	1
b	*Loxophyllum utriculariae*	—	—	1	—	—	—	1	—	—
b	*Bryophyllum* sp.	—	—	—	—	—	1	—	—	—
b	*Dileptus anguillula*	1	2	3	1	4	4	5	6	5
c	*Dileptus americanus*	—	—	1	—	1	—	—	—	1
a	*Plagiopyla* sp.	2	1	—	1	2	2	3	2	6
a	*Trichopelma sphagnetorum*	—	1	1	4	5	4	5	4	3
b	*Drepanomonas* sp.	1	1	—	—	1	1	—	1	—
b	*Kreyella muscicola*	6	—	6	—	—	3	—	—	5
b	*Colpoda cucullus*	—	—	1	2	3	2	3	3	1
a	*Colpoda steinii*	2	2	2	6	5	5	6	3	4
a	*Colpoda inflata*	—	3	1	1	3	3	4	5	6
c	*Frontonia depressa*	—		—	3	—	—	2	1	1
a	*Pseudoglaucoma muscorum*	4	3	4	1	6	4	—	6	1
c	*Tetrahymena rostrata*	—	—	—	1	—	—	2	—	2
b	*Homalogastra setosa*	—	—	—	—	—	1	—	—	4
c	*Saprophilus muscorum*	—	4	—	5	5	3	5	1	5
b	*Cinetochilum margaritaceum*	—	1	—	5	3	6	5	4	3
b	*Cyrtolophosis mucicola*	—	—	1	—	—	—	5	4	2
b	*Cyclidium muscicola*	3	2	4	4	2	3	3	3	5
b	*Cohnilembus fusiformis*	2	—	1	—	—	—	2	1	5
b	*Bryometopus pseudochilodon*	—	—	1	3	—	—	1	—	—
c	*Blepharisma steinii*	—	—	—	—	—	—	3	3	2
c	*Blepharisma hyalinum*	—	—	—	—	—	—	—	6	3
c	*Halteria grandinella*	—	—	—	—	—	—	5	3	3
a	*Uroleptus mobilis*	3	5	6	5	1	3	3	5	4
b	*Uroleptus* sp.	—	—	—	—	—	—	2	2	2
a	*Keronopsis muscorum*	6	5	6	2	3	2	5	6	6
c	*Hemicycliostyla* sp.	—	—	—	—	—	—	—	1	—
a	*Oxytricha pellionella*	3	3	—	5	4	4	6	6	6
c	*Oxytricha setigera*	—	—	—	—	—	—	2	3	1
b	*Gonostomum affine*	—	1	1	5	5	3	5	6	5
c	*Euplotes* sp.	—	—	—	—	—	—	—	—	1
b	*Vorticella* sp.	2	—	3	6	2	6	2	1	4
	Total number of species (TS)	17	19	22	24	21	25	30	30	36
	Total species-incidence (TI)	48	45	57	69	64	75	96	104	113
	Average species-incidence (TI/TS)	2.8	2.4	2.6	2.9	3.0	3.0	3.2	3.5	3.1

give the full list of species arranged in systematic order from the three soils and the number of cultures in which they were recorded in each of the three seasons. At the foot of these tables is given the total number of species recorded from each soil for each season (TS), the total species-incidence (TI) which is the sum of the occurrence of each species in the different cultures for each season, and the average species-incidence (TI/TS) which is the ratio of these two figures. Finally, in Table 4, these figures (the total number of species (TS) and the average species-incidence (TI/TS)) are given separately for the tussock and inter-tussock zones for each of the three soils and each of the three seasons.

TABLE 4. Total Number of Species (TS) and Average Species-incidence (TI/TS) in Tussock (T) and Inter-tussock (I) Zones

Rhizopods

	Taupo (Waiouru)				Tekoa Soil (Bealey)				Omarama Soil (Alexandra)			
	T		I		T		I		T		I	
	TS	TI/TS	TS	TI/TS	TS	TI/TS	TS	TI/TS	TS	TI/TS	TS	TI/TS
Spring	11	2.0	11	2.1	9	1.4	8	1.8	13	1.5	11	2.0
Summer	10*	1.2*	8*	1.5*	12	2.2	10	2.1	15	1.9	11	2.2
Autumn	12	2.0	11	2.1	10	2.5	11	2.3	12	1.9	11	2.0
Total	15	3.86	11	5.45	13	5.0	13	4.77	19	3.79	15	4.6

Ciliates

	Taupo Soil (Waiouru)				Tekoa Soil (Bealey)				Omarama Soil (Alexandra)			
	T		I		T		I		T		I	
	TS	TI/TS	TS	TI/TS	TS	TI/TS	TS	TI/TS	TS	TI/TS	TS	TI/TS
Spring	15	1.86	13	1.5	20	1.75	17	2.0	28	1.9	23	1.9
Summer	20*	1.2	17*	1.35*	20	1.75	16	1.8	30	1.9	21	2.2
Autumn	18	1.8	14	1.7	20	1.9	20	1.85	31	2.0	28	1.8
Total	28	3.0	22	3.0	27	4.0	25	4.0	41	4.25	35	3.97

* The summer Waiouru figures are based on the duplicate cultures and are not therefore strictly comparable. The two duplicate series are treated separately below.

	Rhizopods		*Ciliates*	
	TS	TI/TS	TS	TI/TS
Ta	9	1.4	16	1.4
Tb	7	1.75	17	1.4
Ia	6	1.8	13	1.7
Ib	7	2.1	13	1.9

These tables show that there is marked difference between the ciliate and rhizopod populations.

There are relatively few species of rhizopods involved and they are similar in all three areas. In the Taupo soil the average species-incidence is higher in spring and autumn than in summer, in the Tekoa soil it is lower in spring than in summer and autumn, and in the Omarama soil it is slightly higher in summer than spring or autumn (Table 1). The total species-incidence is slightly higher in the Omarama than in the Tekoa soil and slightly higher in the Tekoa than in the Taupo soil and these differences appear to be chiefly due to differences in the number of species occurring in each area, namely 19 in the Omarama soil, 16 in the Tekoa soil, and 14 in the Taupo soil rather than to differences in the average species-incidence (TI/TS) (Table 2). There is no difference between the tussock and inter-tussock zones in any of the soils but there are clearly few rhizopods in the subsoil (Table 1).

Conversely, the ciliates show a marked difference between the three soils but very little seasonal variation. Again there are few ciliates in the subsoil but there tend to be more species on the average in the tussock zone than in the inter-tussock zone (Table 1). This appears to be due, in the case of the Tekoa and Taupo soils, to an increase in the number of species recorded from the tussock zone samples (TS) rather than an increase in the average species-incidence (TI/TS), and in the Omarama soil to both these factors (Table 4). The differences between the three soils are clearly marked however the results are analysed. There are more species in the Omarama soil than in the other two soils (Table 3) and this is reflected in the average number of species in the cultures (Table 1). But also the average species-incidence (TI/TS) is higher in the Omarama and Tekoa soils than in the Taupo soil (Tables 4 and 3) so that these two factors separate the faunal characteristics of the three soils and give some indication of the activity of the protozoa.

These figures are generally substantiated by the cultural activity of the fauna as indicated in Table 5 where the total incidence of species in the cultures over 10 observations is given, the occurrence of each species on each separate occasion being rated as 1.

These figures suggest that despite the similarity of the average species-incidence the activity of the rhizopods is greater in the Tekoa and Omarama soils than in the Taupo soil. They also indicate that the ciliate activity is far greater in the Omarama and Tekoa soils than in the Taupo soil, confirming the differences shown by the average species-incidence. Overall these figures suggest that the ciliate fauna is much more active than the rhizopod fauna and that on the whole there is greatest protozoan activity in the Omarama soil, less in the Tekoa, and least in the Taupo.

Qualitative

The rhizopod fauna is similar in the three soils. It is listed, with the total incidence of each species, in Table 2. It consists of five

TABLE 5. SPECIES ACTIVITY IN THE CULTURES OVER 10 SEPARATE OBSERVATIONS

(Occurrence of each species on each occasion rated as 1)
T—tussock zone; I—inter-tussock zone; S—subsoil zone.

Rhizopods

	Taupo Soil (Waiouru)				Tekoa Soil (Bealey)				Omarama Soil (Alexandra)			
	T	I	S	Total T + I	T	I	S	Total T + I	T	I	S	Total T + I
Spring	22	26		48	8	12		20	8	12		20
Summer	32	26		58	65	56		121	72	51		123
Autumn	23	25	1	48	31	43	1	74	29	25	1	54
Total	77	77		154	104	111		215	108	88		197

Ciliates

	Taupo Soil (Waiouru)				Tekoa Soil (Bealey)				Omarama Soil (Alexandra)			
	T	I	S	Total T + I	T	I	S	Total T + I	T	I	S	Total T + I
Spring	28	38		66	91	85		176	151	101		252
Summer	68	87		155	135	102		237	163	151		314
Autumn	93	65	2	158	113	102	12	215	177	143	21	320
Total	189	190		379	339	289		628	491	395		886

common soil amoebae, two common proteomyxids, fourteen testacean species belonging principally to the genera *Trinema*, *Euglypha*, and *Difflugia*, and the heliozoan, *Raphidiophrys elegans*. In the Omarama soil there also occurred a *Heleopora* sp. and an *Arcella* sp. Apart from these two species the fauna consists of the most common and widespread species, of the testaceans those best able to survive desiccation, and a fauna which is characteristic of the grassland soils of New Zealand.

The ciliate fauna is very much larger, consisting of 45 species, and very much more diverse. It is listed in Table 3 with the seasonal incidence of each species. Three main ecological groups may be distinguished: first (group a, Fig. 1), the almost omnipresent species such as *Enchelys* and *Colpoda*, small morphologically simple ciliates tolerant of a wide range of environmental conditions (cf. Stout 1955) and with a well developed encystment mechanism; secondly (group b, Fig. 1), the less common species, such as *Lacrymaria* and *Cyrtolophosis*, generally similar to the first group but less well adapted to soil conditions; thirdly (group c, Fig. 1), ciliates such as *Blepharisma* and *Frontonia*, morphologically more highly specialised and commonly with a less efficient encystment mechanism (cf. Stout 1956c) and which are consequently more exacting in their ecological requirements.

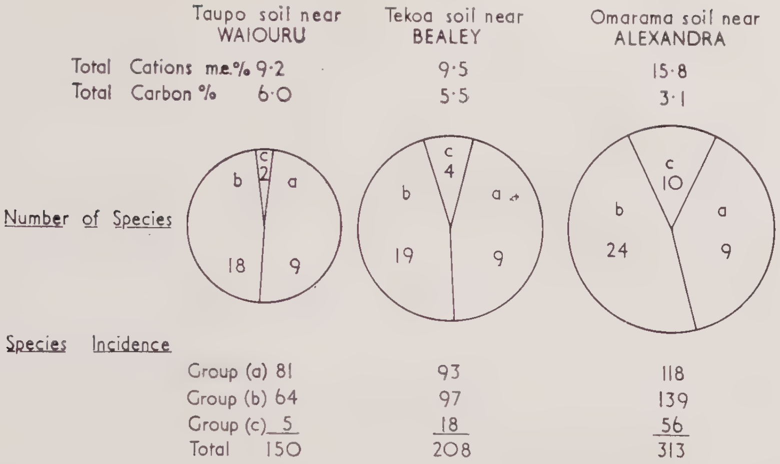

Taupo soil near WAIOURU
Tekoa soil near BEALEY
Omarama soil near ALEXANDRA
Total Cations m.e.% 9·2
9·5
15·8
Total Carbon % 6·0
5·5
3·1
Number of Species
c 2
b 18
a 9
c 4
b 19
a 9
c 10
b 24
a 9
Species Incidence
Group (a) 81
93
118
Group (b) 64
97
139
Group (c) 5
18
56
Total 150
208
313

Fig. 1.—Diagrammatic representation of the relationship between the ciliate fauna of the three soils. The three groups (a, b, c) represent increasing complexity of structure and increasing intolerance of adverse environmental factors.

The distribution of these three groups in the three soils is instructive. The first two groups are found in all three soils but the third group is found chiefly in the Omarama soil. Consequently, the total number of species found in the Tekoa and Taupo soils is roughly the same, viz 32 and 29 respectively, while there are 43 species in the Omarama soil. As shown diagrammatically in Fig. 1 it is this incidence of the more selective ciliate species which chiefly distinguishes the Omarama fauna from the Tekoa and Taupo fauna whereas the difference between the Tekoa and Taupo faunas lies in the average species-incidence. The average species-incidence in the Omarama soil however is also slightly higher than in the Tekoa soil.

These facts suggest that environmental conditions in the Taupo soil restrict the growth of the fauna. There is, however, no indication of what the restrictive factors may be. Possibly it may be due to the microflora. In a previous paper (Stout 1958) attention was drawn to the higher incidence of fermenting bacteria in the Taupo soil. The activity of such bacteria tending to raise the carbon dioxide tension and acidity would have an adverse effect on the fauna (cf. Stout 1956a). Further, *Serratia* was much more common in the Taupo soil than in the Tekoa soil and was not present at all in the Omarama soil. It is known that the pigment formed by this bacterium is toxic to ciliates (Kidder and Stuart 1939).

In the Omarama soil, on the other hand, the occurrence of the more sensitive ciliate species is associated with a much higher base status than in the other two soils and consequently a less acid soil. It seems reasonable to assume that the high base status will tend to have a buffering effect in the micro-environment and that this, associated with the fine crumb structure and low clay content of the Omarama soil will counteract any tendency towards high carbon-dioxide tensions or increased acidity. In this respect, whereas the Tekoa fauna is typical of the ciliate fauna of the moderate to strongly leached yellow-brown earths, the Omarama fauna most closely resembles the faunas of less leached soils derived from basalt or andesite, similarly high in bases.

Apart from the protozoan fauna, there occurred in the cultures the usual collection of microscopic invertebrates. They are listed in Table 6. The only two groups that call for comment are the Turbellaria and the nematode *Criconemoides*.

TABLE 6. INCIDENCE OF NON-PROTOZOAN FAUNA IN THE THREE SOILS
I—spring; II—summer; III—autumn.

	Taupo Soil (Waiouru)			Tekoa Soil (Bealey)			Omarama Soil (Alexandra)		
	WI	WII	WIII	BI	BII	BIII	AI	AII	AIII
Mites		X		X	X			X	X
Collembolans			X					X	
Nematodes			X		X	X	X	X	X
Criconemoides	X	X	X			X			
Rotifers		X	X			X	X		X
Tardigrades	X		X	X	X	X			X
Turbellarians				X				X	
Enchytraeids	X	X	X	X			X		

The Turbellaria (Rhabdocoela) are fully aquatic, ciliated worms less than 1 mm in length, reproducing asexually by fission, and able to form cysts. Ecologically, then, they are very similar to the ciliate protozoa and may be considered a very large ciliate. Because of their size they are not common in soil and are generally found in forest litter or peat. Their occurrence in the Omarama and Tekoa soils is therefore of some ecological significance indicating a relatively favourable niche for an aquatic fauna.

The nematode *Criconemoides* occurs commonly in acid and particularly peaty soils. Its regular occurrence in the Taupo soil, its single incidence in the Tekoa soil, and its non-occurrence in the Omarama soil is therefore also of ecological significance. The occurrence of this nematode in the Tekoa soil may be associated with the previous history of the area, formerly (within the last hundred years) under beech (*Nothofagus cliffortioides*) forest the litter of which is a favourable habitat for the nematode.

DISCUSSION AND CONCLUSIONS

Each of the three soils studied has a typical grassland microfauna and, of the two major protozoan groups studied, it is evident that the ciliate fauna is in each case larger than the rhizopod fauna. This is in agreement with other work done on grassland soils in New Zealand but in conflict with the prevailing opinion (Meiklejohn 1955) that the ciliates are not an important element of the microfauna. The view probably prevails because of the greater morphological complexity of the ciliates and because they are known to be a strictly aquatic group whose activity, it might be thought, is restricted in a "terrestrial" environment. However, the ciliates occurring in soil belong to a large fauna found also in forest litter, moss, and peat, and specially adapted to this environment (cf. Stout 1956b, c). Moreover it is clear that moisture is not the major factor limiting their activity in soil, for the highest number of species, in the present study, was found in the Omarama soil with an annual rainfall of about 20 inches (compared with 48 in. at the Taupo site and 65 in. at the Tekoa site) and a moisture status significantly lower than the other two soils. Indeed the incidence of ciliate species is actually negatively correlated with the moisture status of the soil in each case, the figures % moisture/dry weight ranging from 37–58 (mean 47) for the Taupo soil, 39–58 (mean 47) for the Tekoa soil, and 13–29 (mean 22) for the Omarama soil.

Nor does food seem to be a limiting factor for either group of protozoa in these three soils. For while rhizopods normally increase in numbers with the accumulation of plant material, as in peats or litters, there is no marked difference between the faunas of the three areas despite differences in plant cover. At the Taupo soil site there is almost 100% plant cover, mostly pasture and young *Festuca*, at the Tekoa soil site there is about 90% cover of pasture and mostly young tussock (*Festuca* and *Poa*) and at the Omarama soil site only 50% cover of tussock, mainly old *Poa*. Further there has been heavy grazing of the Omarama site by rabbits, only moderate grazing by sheep at the Tekoa site, and only light grazing by sheep at the Taupo site. Despite these differences in the nature and condition of the plant cover there is no marked effect on the rhizopod fauna which appears to be reduced in each case to a minimal grassland fauna. One factor which may be significant is that although the vegetation is less at the Omarama site there is a higher algal population than at the other two soils (Dr. Flint, pers. comm.) and this would provide a possible food source for the protozoan fauna.

In the case of the ciliates, which are chiefly bacteriophagous, there is no evidence of any great difference in the general character of the bacterial flora quantitatively or qualitatively for the three soils, apart from the incidence of fermenters already noted. This similarity obtains despite quite marked differences in the organic content of the three soils as measured by total carbon figures. Further, Alexandra which might be thought to be the least favourable in this respect actually has the largest ciliate fauna. An instructive example is the distribution of *Tetrahymena rostrata*. This ciliate is an obligate histophage and a facultative parasite of enchytraeid worms (Stout 1954). From the point of view of food, it depends on the turnover of microbial life and the frequent incidence of dead worms, rotifers, nematodes, tardigrades, or even dead ciliates, as much as the incidence of living enchytraeids which in nature are probably only occasionally parasitised. This ciliate was recorded four times from the Omarama soil, once from the Tekoa soil, and not at all from the Taupo soil despite the presence of enchytraeid worms in all three soils, and this seems a clear indication of the far greater microfaunal activity in the Omarama soil. The distribution of the rhabdocoel turbellarian, a strictly aquatic worm and the most exacting in its ecological requirements of the non-protozoan fauna, also supports this view, since it occurred in the Omarama and Tekoa soils but not in the Taupo soil.

REFERENCES

KIDDER, G. W.; STUART, C. A. 1939: Growth Studies on Ciliates. I. The Role of Bacteria in the Growth and Reproduction of *Colpoda*. *Physiol. Zool. 12:* 329-40.

MEIKLEJOHN, J. 1955: Soil Microbiology. Some Microscopical Methods and Results. *J. Quekett micr. Cl. ser. 4; 4:* 171-84.

SINGH, B. N. 1955: Culturing Soil Protozoa and Estimating their Numbers in Soil. *In* "Soil Zoology." Ed. D. K. McE. Kevan, Butterworths Scientific Publications, pp. 403-411.

STOUT, J. D. 1954: The Ecology, Life History and Parasitism of *Tetrahymena* [*Paraglaucoma*] *rostrata* (Kahl) Corliss *J. Protozool. 1:* 211-5.

——— 1955: Environmental Factors affecting the Life History of Three Soil Species of *Colpoda* (Ciliata). *Trans. roy. Soc. N.Z. 82:* 1165-88.

———1956a: Reaction of Ciliates to Environmental Factors. *Ecology 37:* 178-91.

——— 1956b: *Saprophilus muscorum* Kahl, a Tetrahymenal Ciliate. *J. Protozool. 3:* 28-30.

——— 1956c: Excystment of *Frontonia depressa. J. Protozool. 3:* 31-2.

———1958: Biological Studies of Some Tussock-grassland Soils. IV. Bacteria. *N.Z J. agric. Res. 1:* 943-57.

THORNTON, R. H. 1958: Biological Studies of Some Tussock-grassland Soils. I. Introduction, Soils and Vegetation. *Ibid. 1:* 913-21.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

VIII. STREPTOMYCETES

By T. R. Vernon, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(*Received for publication, 19 June 1958*)

Summary

Isolations from the soil of three areas of New Zealand tussock-grassland showed that the streptomycetes form an important part of the soil flora.

The isolates were remarkably uniform and could be placed in four morphological groups.

While there were many similarities between the isolates from the three soils, those from the Taupo and Tekoa soils showed the closest affinities.

From the Omarama soil two groups of streptomycetes which were poorly represented in Taupo and Tekoa soils formed 43% of the population.

Introduction

This paper forms part of a general study of the microfauna and microflora of three tussock-grassland soils. Samples were collected from the three sites previously described (Thornton 1958). Ten separate samples from each site were taken during the spring, summer, and autumn (1956–7). The samples were from the inter-tussock zone of topsoil, 2–4 inches deep.

Methods

Isolations

Isolations were made from soil dilution plates using dextrose casein agar (Jensen 1930) prepared as follows:

Dextrose	2.0 g
Casein dissolved in 10 ml of 0.1N NaOH	0.2 g
K_2HPO_4	0.5 g
$MgSO_4$	0.2 g
Water	1 l
Agar	15.0 g
pH	6.5 7

Cultural

Cultural and morphological characteristics were studied basically on a synthetic medium, Czapek's agar. Non-reproducible media were used only when the cultures failed to produce spores on synthetic media.

Physiological

Carbohydrate utilisation was studied using the basal medium of Pridham and Gottlieb (1948). Carbohydrates were seitz filtered and added to the basal medium at 1% level. Inoculation was by spore suspension. Cultures were incubated for 10–14 days at 27°C.

An indication of the antagonistic properties of the isolates was obtained by the use of agar cylinders 7 mm in diameter cut from 10-day-old plate cultures using Emerson's agar (Gottlieb *et al.* 1948). The test organisms were *Staphylococcus aureus*, *Bacterium coli*, and *Botrytis allii.*

RESULTS

The soil dilution plates showed that the streptomycetes form an important part of the soil flora. The dextrose-casein agar used supports the growth of many soil bacteria and fungi as well as streptomycetes and usually gives figures of the same order as nutrient agar. The results in Table 1 are given for what they are worth, having regard for the limitations of the method employed.

TABLE 1. AVERAGE NUMBER OF ORGANISMS, PER GRAMME OF SOIL FROM THREE SOILS

Soil	No. of Samples	Average Total Organisms (10^6/g)	Average Total Streptomycetes (10^6/g)	Percentage Streptomycetes
Taupo soil (Waiouru)	29	64	37	58
Tekoa soil (Bealey)	30	27	17	62
Omarama soil (Alexandra)	27	39	16	41

One hundred and eighty-two isolates, seventy-one from Taupo soil, fifty-eight from Tekoa soil and fifty-three from Omarama soil were cultured and subjected to morphological examination and various physiological and cultural tests. Examination of these isolates indicated a very restricted streptomycete flora. Most of the cultures (90%) were grey-spored, strongly proteolytic, and strongly chromogenic, producing a dark-brown soluble pigment in organic media. They could be placed in the following morphological groups. These groups correspond to some of the groups suggested by Hesseltine *et al.* (1954).

Group 1. Spirals not present. Sporophore bearing short straight branches.

Group 2. Spirals not present. Sporophore bearing branches either short and straight or long and wavy. Sporophores forming in tufts.

Group 3. Spirals present. Spirals loose and open 2–8 turns usually sinistrorse.

Group 4. Spirals present. Spirals tight and compact 3–6 turns often dextrorse. Spirals sometimes poorly developed, clumped together, and tangled.

Table 2 shows the distribution of the isolates among the four morphological groups.

TABLE 2. PERCENTAGE OF ISOLATES FROM EACH SOIL IN THE FOUR MAIN MORPHOLOGICAL GROUPS

Soil	Morphological Group 1	2	3	4	Not classified
Taupo soil (Waiouru)	1	2	44	49	5
Tekoa soil (Bealey)	1	9	32	56	3
Omarama soil (Alexandra)	26	17	26	27	4

The cultures from Taupo and Tekoa soils clearly show the closest affinities, 93% of the former and 88% of the latter falling into groups 3 and 4. The isolates from the Omarama soil, on the other hand, are spread somewhat wider, 26% falling in group 1 and 17% in group 2. Both these groups are poorly represented in the Taupo and Tekoa soils.

Physiological

Table 3 shows the most common carbohydrate utilisation patterns.

TABLE 3. UTILISATION OF CARBOHYDRATES

Carbohydrate								Percentage of Isolates Showing Each Utilisation Pattern		
Xylose	Lactose	Rhamnose	Raffinose	Mannitol	Inositol	Sorbitol	Inulin	Taupo Soil (Waiouru)	Tekoa Soil (Bealey)	Omarama Soil (Alexandra)
+	+	+	+	+	+	+	+	3.4	4.2	2.6
+	+	+	+	+	+	—	+	18.7	54.6	26.0
+	+	+	+	+	+	—	—	15.3	16.8	15.6
+	+	—	+	+	+	—	+	20.4	0	2.6
+	+	+	—	+	+	—	—	10.2	4.2	2.6
+	+	—	+	—	—	—	—	3.4	0	0
+	—	+	—	+	—	—	—	3.4	0	0
+	+	+	+	+	—	—	+	0	0	7.8
+	+	+	+	+	—	+	+	0	0	5.2

Antibiosis

Under the conditions of test the isolates did not exhibit notable antagonistic properties.

Table 4 summarises the antagonistic properties of the isolates from the three soils.

TABLE 4. ANTAGONISTIC PROPERTIES OF ISOLATES AGAINST THREE TEST ORGANISIMS

Test Organism			Percentage of Isolates Showing Antagonistic Properties		
S. aureus	*B. coli*	*Botrytis allii*	Taupo Soil (Waiouru)	Tekoa Soil (Bealey)	Omarama Soil (Alexandra)
—	—		37	57	37
+	—	—	0	5	3
+	+	—	2	2	0
+	+	+	4	5	6
+	—	+	15	0	14
—	+	—	10	13	6
—	+	+	0	2	11
—	—	+	32	15	23

In view of the confused state of the taxonomy of the streptomycetes it seemed advisable with this restricted flora to sort out the isolates into groups rather than attempt a more precise classification.

The following groups, which take into account the basic micromorphology, spore colour, and certain physiological properties, account for 87% of the isolates from the three soils.

Group A. Sporophores usually short: sometimes forming long strands, branches bearing loose open spirals. Spirals, 2–8 turns usually sinistrorse. Spore colour, en masse, shades of grey, usually mouse-grey or dark grey. Gelatin liquefied. Dark-brown soluble pigment on organic media.

The 61 isolates which fell into this main group could be further divided as follows:

A1. Utilising all carbohydrates under test except sorbitol—33 isolates.

A2. As above but showing slight utilisation of sorbitol—28 isolates.

Group B. Sporophores usually short, sometimes forming long strands. branches bearing tight compact spirals. Spirals 3–6 turns often dextrorse. Spore colour, en masse, shades of grey, light grey to dark grey.

Gelatin liquified. Dark-brown soluble pigment on organic media.

The 44 isolates in this group could be divided on the basis of carbohydrate utilisation as follows:

B1. Utilising all carbohyrates under test.

B2. No utilisation of sorbitol.

B3. No utilisation of sorbitol or inulin.

B4. No utilisation of sorbitol or rhamnose.

Group C. Sporophores with short branches bearing poorly formed spirals. Spirals clumped together and tangled. Spore colour, en masse, shades of grey. Gelatin liquefied.

C1. Dark-brown soluble pigment on gelatin and other organic media. Carbohydrate utilisation variable.

C2. No soluble pigment on gelatin and other organic media. Carbohydrate utilisation variable.

Group D. Sporophore with straight branches. No spirals. Spores formed in straight chains.

D1. Gelatin liquefied but no soluble pigment. In some organic media an orange-brown soluble pigment is formed. Spore colour, en masse, white to light grey.

D2. Gelatin liquefied. Dark-brown soluble pigment in gelatin. Reddish-brown soluble pigment in some organic media. Spore colour, en masse, grey.

Group E. Sporophore branched. Branches usually short and straight but sometimes long and wavy. No spirals. Sporophores in tufts. Gelatin liquefied. No soluble pigment in gelatin or other organic media. Spore colour, en masse, white to light grey.

TABLE 5. Number of Isolates from Each Soil Grouped According to Main Morphological and Physiological Characteristics

Soil	Group										
	A1	A2	B1	B2	B3	B4	C1	C2	D1	D2	E
Taupo soil (Waiouru)	17	13	2	4	7	5	5	6	—	—	—
Tekoa soil (Bealey)	10	12	5	14	5	—	1	3	—	1	-
Omarama soil (Alexandra)	6	3	—	—	2	—	3	5	13	8	10

Twenty-two cultures did not fit into these groups and of these 9 failed to produce aerial mycelium after the initial isolation.

Discussion

The streptomycete flora is clearly restricted and there are marked similarities in the types present in the three soils. The absence of many species commonly found in soil suggests some limiting factors. Nothing that has been done in the present investigation indicates what these factors may be.

The presence in considerable numbers in the Omarama soil of two groups very poorly represented in Taupo and Tekoa soils is noteworthy.

Acknowledgement

The technical assistance given by Mrs. J. M. Rainey and Miss P. Fulton is gratefully acknowledged.

REFERENCES

GOTTLIEB, D.; BHATTACHARYYA, P. K.; ANDERSON, H. E.; CARTER, H. E. 1948: Some Properties of an Antibiotic Obtained from a Species of Streytomyces. *J. Bact. 55:* 409-17.

HESSELTINE, C. W.; BENEDICT, R. G.; PRIDHAM, T. G. 1954: Useful Criteria for Species Differentiation in the Genus Streptomyces. *Ann. New York Acad. Sci. 60:* 136-51.

JENSEN, H. L. 1930: The Genus Micromonospora. *Proc. Linn. Soc. N.S.W. 45:* 231-48.

PRIDHAM, T. G.; GOTTLIEB, D. 1948: The Utilisation of Carbon Compounds by Some Actinomycetales as an Aid for Species Determination. *J. Bact. 56:* 107-14.

THORNTON, R. H. 1958: Biological Studies of Some Tussock-grassland Soils. I. Introduction, Soils and Vegetation. *N.Z. J. agric. Res. 1:* 913-21.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

IX. ALGAE : PRELIMINARY OBSERVATIONS

By E. A. FLINT*

(*Received for publication, 19 June 1958*)

Summary

A marked resemblance has been found between the ecology of the major groups of algae in the soil of three tussock grasslands and the occurrence of these groups in the soils of other temperate countries. The algal growth has been greatest on Omarama soil from near Alexandra where Bacillariophyceae (diatoms) and Cyanophyceae (blue-green algae) were associated with a high pH and a high concentration of nutrient salts. The poorest algal growth occurred on Tekoa soil from Bealey where the higher vegetation had relatively recently changed from forest to grassland. Chlorophyceae (green algae), particularly the desmids, were common on Taupo soil from Waiouru. In all samples the algae were almost confined to the surface layers of the soil but considerable growth developed when soil from a depth of 1¼ in. (3 cm) was cultured, suggesting the presence of dormant cells in the lower layers of the soil.

INTRODUCTION

Although the rivers and lakes of New Zealand are numerous and varied, relatively little is known of the algae living in them, and as pointed out elsewhere (Chapman *et al.* 1957) since Nordstedt's account in 1888 which included a summary of earlier work, the study of freshwater algae has been almost neglected. Apart from the fact that Nordstedt (1888) examined some material which was collected from damp earth, it seems that the true soil algae have received no attention at all.

In 1956, preliminary work on the algae in the soils of three tussock grasslands was started with a view to identifying the commoner species, and determining their frequency, seasonal periodicity, and vertical distribution at the time of sampling. By using different methods of culture, it was hoped that evidence might be obtained of the existence of other species in the soil.

Full details of the three soils are given by Thornton (1958). It should be noted that the results for the physical and chemical analyses refer to soil at 2–4 in. depth and they may not apply to the soil at the surface. No information is available on the concentration of nitrates in these soils.

METHODS

The cores of soil, which were collected in an undisturbed state with a cylindrical soil sampler, measured 4 in. diameter by $3\frac{1}{4}$ in. Triplicate samples were taken from each of the three sites at the following times (some allowance was made for the difference in the latitude of the sites)—October or November 1956, January or February 1957, April or May 1957, October or November 1957; trial experiments were made on samples of Taupo soil from Waiouru collected in August 1956. All cores were conveyed to the laboratory, intact, in the samplers and examined two to four days after collection. The surface of most of the cores of Taupo soil from Waiouru and of Tekoa soil from Bealey was covered with a dense growth of grasses, herbs, mosses, and a few lichens. Among the mosses and among the basal leaves of grasses were found species of *Cylindrocystis, Mesotaenium, Hantzschia, Nostoc, Phormidium,* and *Stigonema.* The cores of Omarama soil from Alexandra usually had few plants on them (except those collected in spring 1957, when many angiosperms were present) and they were either bare or covered with small stones and showed the effect of frost action. Macroscopic colonies of *Nostoc* were found on some cores from each of these soils.

---

* Working under a D.S.I.R. grant at the University of Canterbury.

The methods used for the examination and culture of the algae are based on those described by Lund (1945, 1947). For the direct observation of the soils, small duplicate samples were scraped from the surface of each core with a sterile triangular needle and similar samples from a depth of $1\frac{1}{4}$ in. (3 cm) were removed with a sterile cork borer inserted horizontally into a core (these are subsequently referred to as surface and subsurface samples respectively). Each sample was mounted in sterile distilled water under a coverslip, 2 × $\frac{3}{4}$ in., and, using 1/6 objective and x8 eyepiece, the algae were counted in four horizontal traverses which were made along the length of the coverslip.

Other samples, taken from the surface and subsurface by the same methods, were treated as follows:

(a) Soil moistened with sterile distilled water was placed on a slide in a moist chamber and examined daily for several days.

(b) Small samples of soil were mixed with sterile distilled water and poured on to plates of agar (1–2%) dissolved in 0.05% Benecke solution for which de-ionised water was used. Benecke solution contains NH_4NO_3, 0.2 g; K_2HPO_4, 0.1 g; $MgSO_4.7H_2O$, 0.1 g; $CaCl_2.6H_2O$, 0.1 g; $FeCl_3.6H_2O$, one drop of 1% solution; water 1000 ml. All culture media were prepared with de-ionised water which was obtained by passing distilled water through a column of Zeo Karb 225 and Deacidite G. This mixed bed of resins was made according to the specifications given by British Drug Houses Ltd. in "Ion Exchange Resins" 2nd edition, Poole, England.

(c) Enriched cultures consisted of soil spread to a depth of about $\frac{1}{2}$ in. on the bottom of covered petri dishes and moistened with 0.05% Benecke solution. Sterile cover slips were placed on the soil and the algae which grew on them could be examined easily. Liquid cultures, which are believed to favour the growth of aquatic algae at the expense of soil algae, were not used.

(d) Uni-algal cultures, essential for the identification of some species, were grown in soil/water medium (Pringsheim 1946), on Detmer's medium as modified by Bold (1942), or on a 0.05% Benecke medium. Soil extract (De 1939) was prepared from cores of Omarama soil and added to the last two media. All cultures, illuminated with indirect daylight, were grown at a temperature varying between 4° and 35°c. Preparations of diatoms made according to the methods used by Lund (1945), were mounted in Styrax medium.

RESULTS

Frequency of Algae on the Surface at Time of Sampling

It is now well-known that the population of algae varies with the kind of soil and it is evident from these preliminary observations that there are also well-marked local differences, both qualitative and quantitative, between the algae in the two surface samples which were removed from each core of soil. The methods used in determining the frequency of living algae in the soil are subject to certain limitations so that the results they give are only approximately correct. Variation in the texture of the soils inevitably leads to differences in the volume of the very small samples which are mounted on a slide. There is also difficulty in defining the algal unit since some species occur as unicellular organisms, others are colonial and contain a definite or indefinite number of cells, and others develop a filamentous habit which may be simple or branched, solitary or aggregated into bundles of filaments. It has been necessary to ignore these differences and to regard all forms of plant body as individuals, irrespective of their exact structure. In spite of these discrepancies, some general conclusions can be drawn, but until much more progress has been made with the systematic part of the work, they must be limited to a consideration of the major groups of algae and any reference to genera and species must be deferred.

The algae were most numerous in the Omarama soil from near Alexandra (except in autumn when more individuals were found in Taupo soil from Waiouru) and they were always very rare in the Tekoa soil from Bealey (see Table 1). Chlorophyceae (green algae) were dominant in 66% of the samples of Taupo soil and always more common there than elsewhere; they were usually present in the Omarama soil but never common in it. The distribution of Bacillariophyceae resembled that of Cyanophyceae, both occurring most frequently in the Omarama soil with their greatest development in spring and summer. Both these groups were present in Taupo soil but never common except in autumn 1956 when Cyanophyceae became more common there than in either of the other soils. Xanthophyceae (yellow-green algae) were rarely recorded by direct observation and no comments can be made on their distribution.

Frequency of Algae at $1\frac{1}{4}$ in. Depth at Time of Sampling

In spite of the fact that a hundred mounts were examined by direct observation, only six living individuals were found in the sub-

TABLE 1. FREQUENCY OF ALGAE ON SURFACE OF SOIL: AVERAGE NO. OF LIVING INDIVIDUALS SEEN IN A TRAVERSE; ITALICS INDICATE THE DOMINANT GROUP IN A SAMPLE

sample	Chlorophyceae			Cyanophyceae			Bacillariophyceae			Total No. of Individuals in 24 Traverses
	I	II	III	I	II	III	I	II	III	
Taupo soil (Waiouru)										
date 16/ 8/56	2	*5*	*5*	0	2	0	*3*	2	2	166
19/10/56	*5*	*16*	5	4	2	*14*	0	9	0	457
7/ 2/57	1	9	3	0	2	*5*	0	1	0	184
4/ 5/57	*8*	*10*	*3*	3	7	1	1	3	1	317
28/10/57	*7*	1	*8*	1	1	2	2	*4*	3	129
Tekoa soil (Bealey)										
24/11/56	0	1	1	0	0	1	0	0	0	18
26/ 1/57	0	0	0	0	1	0	0	0	0	9
18/ 4/57	1	1	1	0	1	1	0	1	0	42
10/11/57	1	0	0	0	0	0	1	0	0	11
Omarama soil (Alexandra)										
24/11/56	1	8	0	*8*	18	4	5	*29*	*5*	634
30/ 1/57	1	1	1	*7*	*17*	5	0	11	*35*	635
24/4/57	*7*	1	1	3	1	1	2	1	*3*	148
7/11/57	1	1	*8*	0	0	*8*	2	*12*	5	275

surface samples, four being in the Tekoa soil and one in each of the other soils.

Cultures of the Surface Samples of Soil

The occurrence of the algae in the enriched cultures seem to present a basic pattern similar to the one seen by direct observation, but since more than half the cultures have been growing for less than a year, it is premature to draw final conclusions on the distribution of the algae in them.

In general, algae were common more often in cultures of Taupo and Omarama soils while the poorest growth occurred in Tekoa soil. Chlorophyceae, which were most numerous and the dominant group in the cultures of Taupo soil, were less common in cultures of Omarama soil and rare in those of Tekoa soil. In contrast to this, diatoms and Xanthophyceae were more frequently common in cultures from Omarama soil than elsewhere, and the distribution of Cyanophyceae presented some unexpected features in that they were more common in the cultures from Taupo and Tekoa soils and poorly represented in Omarama soil.

Cultures of the Subsurface Samples of Soil

The fact that algae became common in the cultures of soil from $1\frac{1}{4}$ in. depth is strong evidence of the presence of dormant cells or spores at this depth. Algae were particularly common in Omarama soil where the growth of Xanthophyceae was conspicuous, but diatoms, Cyanophyceae, and Chlorophyceae, were decreasingly common in this soil. Although Chlorophyceae and Xanthophyceae were not often common and Cyanophyceae never common in the other subsurface cultures, there are indications that in Taupo soil, Xanthophyceae were a little more common in the subsurface than in the surface cultures.

Seasonal Periodicity

The evidence suggests that the seasonal periodicity which is so characteristic of algae in fresh-water habitats does not occur among algae in these soils. The well defined maximum of diatoms and Cyanophyceae in Omarama soil in the spring 1956 was not repeated in the following spring, but the cores were not strictly comparable since the growth of angiosperms on them was much thicker in the second than in the first spring.

DISCUSSION

The algae found in these uncultivated grassland soils resemble those in other parts of the world where three ecological groups have been distinguished among terrestrial algae (Fritsch 1936):

(a) subterranean community, algae occurring between particles of soil,

(b) surface community, algae on or immediately below the soil surface,

(c) aerial community, algae epiphytic on tree trunks or other plants, on stones and rocks.

These communities are not always well defined but tend to merge into one another and they vary qualitatively and quantitatively with the characteristics of the habitat in which they grow. The fact that algae were abundant in the Omarama soil and rare in Tekoa soil, that Chlorophyceae were dominant in Taupo soil but diatoms and Cyanophyceae dominant in Omarama soil, that Xanthophyceae were common but Cyanophyceae relatively rare in the subsurface cultures, can probably be related to the ecological conditions of the soils.

The significance of some of these factors has been studied in Europe and America. Stokes (1940) increased the population of algae in an experimental plot by applying lime (with and without minerals and ammonium sulphate) but he found that addition of organic matter to a plot depressed the growth of algae. In a culture experiment in which the pH varied between 4.2 and 7.6, Stokes obtained the best growth at pH 7.4–7.6, while others have recorded a maximum growth of algae, in nature, at pH 8.2 (John 1942; Lund 1945, 1947). Other factors associated with a richer population of algae in the soil are a higher percentage base saturation, the presence of nitrates, a concentration of available phosphates above 0.025% (Petersen 1935; Lund 1945, 1947), and a water content between 40% and 60% of the moisture-holding capacity of the soil (Stokes 1940; Tchan and Whitehouse 1953). The fact that algae were more numerous in Omarama soil than in Taupo and Tekoa soils may be associated with the higher pH, the higher percentage base saturation, the higher concentration of available phosphates, and the lower percentage of organic carbon in Omarama soil. (See Thornton 1958, Table 2.) Similarly, an increase in the concentration of available nutrients under alkaline conditions are known to favour the growth of diatoms, Cyanophyceae, and Xanthophyceae, all of which were numerous in Omarama soil (Petersen 1935; John 1942; Lund 1945, 1947). In contrast to these groups, some species of Chlorophyceae are characteristic of acid soils which are poor in nutrients (John 1942; Lund 1947), and the presence of this group in Taupo soil may be associated with the acid condition and the medium percentage base saturation in this soil.

It has been found elsewhere (Petersen 1935; John 1942; Lund 1947) that forest soils are poorer than pasture soils in both individuals and species. John (1942), who studied seven forest soils (pH 6.6 or higher), found that each soil contained an average of 15 species (the range varying between 10 and 22 species), whereas the six pasture soils she examined (pH 6.2 or higher) contained an average of 27 species (the range varying between 19 and 40 species in a soil). Lund (1947) quotes Esmarch's results which are further evidence of this fact. It is possible that the scarcity of algal cells in the Tekoa soil may be the result of the history of this habitat, the grassland of which was derived relatively recently from a forest of *Nothofagus cliffortioides* and is growing on a soil in which the percentage base saturation is low and the organic matter has accumulated at the surface. These conditions seem to be unfavourable to the growth of soil algae.

It is generally agreed that the greatest population of algae occurs at the surface of the soil and that there is a decrease in the number of species and individuals with increasing depth (Petersen 1935; Lund 1945, 1947; Willson and Forest 1957). The results of the present study agree with these observations. The community of subterranean algae which has been found at considerable depths (Moore and Carter 1926) includes three elements (Fristch 1936):

(a) a few species, most of them members of Xanthophyceae, which appear to be intolerant of strong light,

(b) a group which is common in the surface community,

(c) a casual element consisting of forms which are usually rare in the soil, whose spores may have been carried by wind from aquatic habitats.

The presence of these three elements has stimulated much research on the growth of algae in the dark and in cultures of organic media (Petersen 1935). Some work has been done on the penetration of light (Tchan and Whitehouse 1953) and on the mechanical agents and the significance of the texture of the soil in the passage of algae to considerable depths (Petersen 1935; Tchan and Whitehouse 1953). Although

John (1942) found that the Cyanophyceae were commoner in cultures of surface soil than in those from the deeper layers, it is possible that the relatively rich growth of this and other groups in the subsurface cultures from Omarama soil and their poor growth in similar cultures from Taupo or Tekoa soils may be partly due to differences in the texture of the soils and in the density of the higher plants and their associated root systems. These factors and the reduction in the intensity of light may be associated with the relative abundance of Xanthophycae in the subsurface cultures of Omarama soil.

ACKNOWLEDGEMENTS

The writer is indebted to the Director of the Soil Bureau through whom a D.S.I.R. research grant was made available for this work, to Dr. R. H. Thornton who collected the samples of soil, to Professor W. Philipson in whose Department at the University of Canterbury the work was carried out, and to Mr. H. Lintott who, in emergencies, maintained the cultures.

REFERENCES

BOLD, H. C. 1942: The Cultivation of Algae. *Bot. Rev. 8:* 69-138.

CHAPMAN, V. J.; THOMPSON, R. H.; SEGAR, E. C. M. 1957: A Check List of the Freshwater Algae of New Zealand. *Trans. roy. Soc. N.Z. 84:* 695-747.

DE, P. K. 1939: The Role of Blue Green Algae in Nitrogen Fixation in Rice Fields. *Proc. roy. Soc. B. 127:* 121-39.

FRITSCH, F. E. 1936: The Role of Terrestrial Algae in Nature. *Essays in Geobotany in honour of W. A. Setchell:* 195-217. University of California.

JOHN, R. P. 1942: An Ecological and Taxonomic Study of the Algae of British Soils. I. The Distribution of the Surface-growing Algae. *Ann. Bot. Lond. n.s. 6:* 323-49.

LUND, J. W. G. 1945: Observations on Soil Algae. I. The Ecology, Size and Taxonomy of British Soil Diatoms. *New Phytol. 44:* 196-219.

——— ———1947: Observations on Soil Algae. II. Notes on Groups other than Diatoms. *Ibid. 46:* 35-60.

MOORE, G. T.; CARTER, N. 1926: Further Studies on the Subterranean Algal Flora of the Missouri Botanical Garden. *Ann. Mo. bot. Gdn 3:* 101-40.

NORDSTEDT, O. 1888: Fresh Water Algae Collected by Dr. S. Berggren in New Zealand and Australia. *K. svenska VetenskAkad. Handl. 22 (8):* 1-98.

PETERSEN, J. B. 1935: Studies on the Biology and Taxonomy of Soil Algae. *Dansk. bot. Ark. 9:* 1-183.

PRINGSHEIM, E. G. 1946: "Pure Cultures of Algae". C.U.P.

STOKES, J. L. 1940: The Influence of Environmental Factors upon the Development of Algae and other Micro-organisms in Soil. *Soil Sci. 49:* 171-84.

TCHAN, Y. T.; WHITEHOUSE, J. A. 1953: Study of Soil Algae. II. The Variations of the Algal Population in Sandy Soils. *Proc. Linn. Soc. N.S.W. 78:* 160-70.

THORNTON, R. H. 1958: Biological Studies of Some Tussock-grassland Soils. I. Introduction, Soils and Vegetation. *N.Z. J. agric. Res. 1:* 913-21.

WILLSON, D.; FOREST, H. S. 1957: An Exploratory Study on Soil Algae. *Ecology, 38:* 309-13.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

X. EARTHWORMS

By K. E. Lee, Soil Bureau, Department of Scientific and Industrial Research, Wellington.

(Received for publication, 19 June 1958)

Summary

Earthworms were collected under tussock grassland (*Festuca novae-zelandiae* dominant) from a Taupo hill soil at Waiouru, a Tekoa steepland soil at Bealey and an Omarama steepland soil near Alexandra.

Although the vegetation was similar at the three sites, geographical separation and differences in the history of the vegetation have been most important in influencing the earthworm fauna.

Higher earthworm populations were observed beneath tussock plants than beneath shorter vegetation or bare ground between tussock plants. This is attributed to protection of the soil from frost by tussock plants.

The constitution of the earthworm fauna at each of the sites is compared with that of soils of adjacent sites; similarities and differences are noted and discussed.

Experimental Data

Earthworms were collected from Waiouru in March 1953, and from Bealey and Alexandra in October 1953, at the sites described by Thornton (1958). Specimens were collected by hand in the field, and stored in 70% ethyl alcohol until identified.

The following species were identified from the sites:—

Waiouru

Fam. Megascolecidae

Rhododrilus albidus Lee
Rhododrilus robustus Lee

Fam. Lumbricidae

Lumbricus rubellus Hoffmeister

Bealey

Fam. Lumbricidae

Allolobophora caliginosa (Savigny)

Alexandra

Fam. Megascolecidae

Eodrilus paludosus (Beddard)
Maoridrilus dissimilis (Beddard)

Fam. Lumbricidae

Allolobophora caliginosa (Savigny)

Waiouru

The site at Waiouru is on a hillside, above the east bank of a small stream, which runs from north to south. The stream valley has moderately steep sides and a wide flat floor, due to in-filling by pumice washed down from the slopes. The stream is deeply incised, with nearly vertical banks 10 ft to 15 ft high, in the flat valley floor.

Commencing at a point on the ridge, at an elevation of about 3,500 ft, overlooking the site described by Thornton, two transects were made, one east to west and the other south-east to north-west, down

N.Z. J. agric. Res. 1: 998–1002.

the valley side, across its floor, and up to the crest of the ridge west of the stream. The dominant tussock plants were *Danthonia rigida* and *Festuca novae-zelandiae*. Earthworms were collected from square-foot samples under each of the dominant tussocks at five sites on each transect—on the ridge top, half way down the valley side, on the valley floor, half way up the west side of the valley, and on the top of the western ridge.

Earthworms were found only in the topsoil (in the top 6 in. to 8 in.) and were more numerous under *D. rigida* than under *F. novae-zelandiae*. Populations ranged from about 100,000 per acre to about 300,000 per acre, based on square-foot samples and were lower on the western than on the eastern side of the valley. Only one specimen of *Lumbricus rubellus* was collected; all the remaining specimens were *Rhododrilus albidus* and *R. robustus*.

R. albidus and *R. robustus* are the dominant earthworms of yellow-brown pumice soils in the area east of National Park and Lake Taupo. They are not confined to tussock grasslands, but occur in soils under forest, scrub, and tussock. Their wide distribution and lack of well defined habitat preferences are atypical of New Zealand Megascolecidae. The yellow-brown pumice soils are formed from volcanic ash, much of which has been erupted in cataclysmic showers of hot pumice within the last 1,700 years. Earthworms would have been destroyed by the hot pumice and the soils formed from the pumice have been populated by migration of species from surrounding unaffected areas, during very recent times. Because of the coarse physical nature of much of the pumice, the low clay content of soils derived from it, and a tendency for the soils to dry out in summer, most earthworms apparently find yellow-brown pumice soils inhospitable, and those few species that can tolerate the conditions have been able to spread widely and have occupied ecological niches from which, in other circumstances, they would probably have been excluded by other species. Lee (in press) has discussed more fully the earthworm fauna of yellow-brown pumice soils and the reasons for its peculiarities.

Lumbricus rubellus is a common earthworm in New Zealand pastures. The Waiouru site and its surroundings have some exotic pasture grasses and the area is used for extensive grazing of sheep. *L. rubellus* must have entered the area as a result of disturbance of the vegetation by farming, but most of the plant cover is tussock and *L. rubellus* has not displaced the indigenous *Rhododrilus* spp.

Bealey

At Bealey, the site is on a steep hillside, overlooking the south bank of the Waimakariri River. The vegetation was beech (*Nothofagus menziesii*) forest, which was cleared about 100 years ago. The present plant cover is predominantly a mixture of exotic pasture grasses and tussock (*Festuca novae-zelandiae*). Beech forest remains close to the site. Sheep graze on the site and the surrounding country.

Allolobophora caliginosa was the only species collected from the Bealey site. This is the most common introduced lumbricid in New

Zealand pastures. Lee (in press) has discussed the sequence of changes in the constitution of the earthworm fauna following replacement of indigenous vegetation by pasture; the final stage of the sequence is reached when earthworm populations consist entirely or almost entirely of *A. caliginosa,* and the dominance of this species at the Bealey site, combined with the absence of indigenous species, may be taken as an indication that the soil fauna has ceased to be influenced appreciably by the indigenous vegetation. Indigenous earthworms are found in the adjacent beech forest, and the same species have been found under snow tussock at Porter's Pass, so the absence of indigenous species at Bealey is not due to the lack of a suitable species in an adjacent area to colonise the tussock soils.

Alexandra

The Alexandra site is in a small, shallow basin on a moderately steep hillside. The dominant tussock species are *Poa caespitosa* and *Festuca novae-zelandiae.* On the ridges bounding the basin there is much bare ground, with patches of scabweed (*Raoulia* sp.) and some tussocks. Tussock grassland was formerly more extensive in the vicinity and has been modified and reduced in area by fire and grazing.

Soil-moisture levels and the density of plant cover are critical factors limiting the distribution and activities of earthworms in Omarama soils. At the sampling site there is apparently sufficient moisture and protection from desiccation in the immediate vicinity of tussock plants for earthworms to survive, but between tussocks and under bare ground earthworms are rare or absent. A series of samples was taken in a line across a large patch of scabweed (about 3 ft in diameter), with tussock plants growing up through the mat formed by the scabweed. Under scabweed alone, where there was about 1 in. of humified soil under the plant cover, no earthworms could be found; under a small *Poa caespitosa* tussock (about 1 in. diameter at ground level) growing in the scabweed patch, where the humified layer was 3 in. to 4 in. deep, no earthworms were found, but a grass grub (*Odontria* sp.) was collected from the tussock root zone; under a larger *P. caespitosa* (about 4 in. diameter at ground level) growing in the scabweed patch, where the humified layer was about 8 in. deep, specimens of *Allolobophora caliginosa, Maoridrilus dissimilis,* and *Eodrilus paludosus* were collected from directly under the tussock at depths of 2 in. to 8 in. Under large tussocks, not surrounded by scabweed, the same species were found at depths of 2 in. to 8 in. No earthworms could be found in the subsoil at the Alexandra site, but on an adjacent shaded, and therefore cooler and moister, slope with easterly aspect, *Festuca* tussock was more abundant and worms were more numerous, with *Octochaetus multiporus* appearing in the subsoil at 6 in. to 18 in.

Allolobophora caliginosa has probably been introduced as a result of farming. Only a small amount of interference with the indigenous vegetation is necessary to allow exotic lumbricids to become established. It seems to be only necessary for sheep to graze on an area for lumbricids to appear, and it may be that earthworm cocoons are carried about on the hoofs of sheep and fall off as the sheep move about. *A. calliginosa* is rare at Alexandra and indigenous earthworms are dominant; the situation is very different from that at Bealey, where *A. caliginosa* is dominant and there are no indigenous species.

DISCUSSION

There are considerable differences between the earthworm faunas of the three sites. The sites are widely separated geographically, and since indigenous New Zealand earthworms are generally distributed only within small areas, it would not be expected that the same indigenous species would be present.

Although there is usually no close systematic relationship between widely separated earthworm populations, species that are similar in their ecological preferences and often in their general morphology are frequently found occupying analogous ecological niches in widely separated localities where the vegetation and other environmental conditions are similar. At Waiouru and Alexandra there is a tendency toward concentration of earthworms under tussock plants, with few earthworms between plants. This is probably due to the protection from extremes of temperature afforded the soil by tussock plants. Gradwell (1955) has noted that diurnal temperature ranges at 1 in. depth at Molesworth Station were approximately halved where the ground surface was covered or shaded by tussock. Similar effects would be expected at the present sites, and would be more important at Alexandra, where rainfall is lower than at Waiouru and high average temperatures with little rain are common in summer.

Subsoil earthworms are absent at the three sites, but the reasons for their absence differ. Yellow-brown pumice soils have a restricted earthworm fauna, comprised of topsoil species and lacking subsoil species. (See discussion of yellow-brown pumice soils in Lee (in press).) At Bealey, indigenous species are absent, and the only earthworm present is *Allolobophora caliginosa*, an introduced lumbricid which lives in the topsoil, close to the surface. At Alexandra, subsoil species are abundant under *Festuca novae-zelandiae* at a neighbouring site on a shaded slope, where the soil is moister, and their absence from the sampling site probably results from its exposure to the sun and consequent lower soil-moisture levels.

The earthworm fauna of New Zealand consists almost entirely of species that live in forest soils. There are few, if any, species peculiar to tussock-grassland soils, and the indigenous species that are found in any particular area of tussock grassland soils are usually derived from adjacent forested areas, or have spread from forested areas across extensive tussock grasslands. This leads to an earthworm fauna consisting of a few species, each widely distributed, contrasting with the large number of species, each occupying only a small area, usually found in forested soils. (For a full discussion of this point, see Lee (in press, part 3).)

The Waiouru fauna is fairly typical of yellow-brown pumice soils. There are four species of *Rhododrilus*, one of *Diporochaeta*, and one of *Megascolides*, any one or more of which can be found at almost any locality in the yellow-brown pumice soils. The Bealey fauna is atypical of South Island tussock grassland and shows strongly the impress of pastoral development. The Alexandra fauna, though slightly modified by the effects of farming and grazing, is fairly typical of Omarama soils, but lacks subsoil species because of the northerly aspect and consequent exposure to the sun of the sampling site; partial denudation of the site by grazing animals may also have contributed to a reduction in the fauna.

REFERENCES

GRADWELL, M. W. 1955: Soil Frost Studies at a High Country Station. II. *N.Z. J. Sci. Tech. B37 (3)*: 267-75.

LEE, K. E. (in press): The Earthworm Fauna of New Zealand. *N.Z. Dep. sci. industr. Res. Bull. 130.*

THORNTON, R. H. 1958: Biological Studies of some Tussock-grassland Soils. I. Introduction, Soils and Vegetation. *N.Z. J. agric. Res. 1*: 913-21.

BIOLOGICAL STUDIES OF SOME TUSSOCK-GRASSLAND SOILS

XI. THE RESPONSE OF A TUSSOCK PLANT, *Festuca novae-zelandiae,* TO FERTILISERS

By T. M. MORRISON, Soil Bureau, Department of Scientific and Industrial Research, Wellington.

(Received for publication, 19 June 1958)

Summary

Fertiliser experiments with *Festuca novae-zelandiae* were carried out in pots, under glasshouse conditions, with Tekoa steepland soil from Bealey. Tussock plants responded to applications of phosphorus and, to a less extent, of potassium and sulphur. Phosphorus in combination with nitrogen gave a four- to five-fold increase in crop yields over one year. Yields were further increased by additions of potassium and sulphur.

INTRODUCTION

There is in New Zealand a growing importance attached to the development of some tussock grasslands for increased agricultural production. Fertiliser requirements and the establishment of introduced grass and clover species have been studied, but there appear to be no published records of the response to fertilisers by tussock plants themselves.

SOIL

Topsoil of Tekoa steepland soil was taken from Bealey at the site used throughout a series of microbiological studies (Thornton 1958). A chemical analysis of this soil is cited by Thornton (1958). An additional analysis by Miss K. M. Slocombe showed exchangeable potassium to be at a medium level (0.45 m.e.%).

METHODS

Seeds of *Festuca novae-zelandiae* were sown in washed silica sand after 15 days stratification at 5°C and seedlings were transplanted, two to each pot, as soon as possible after germination. The soil was contained in 4 in. clay pots holding 354 g air-dry soil.

Nitrogen, phosphorus, potassium, and potassium plus sulphur were added alone, and in combination, to the pots as follows:

Element	Fertiliser	Wt. per pot (g)	Equiv. per acre
N	NH_4NO_3	0.23	80 lb N
P	$NaH_2PO_4.2H_2O$	0.979	1 ton superphosphate
K	KCl	0.219	240 lb K_2SO_4
K + S	K_2SO_4	0.252	240 lb K_2SO_4

Distilled water was used throughout for watering and four replicates were set up for each treatment. Pots were randomised as to

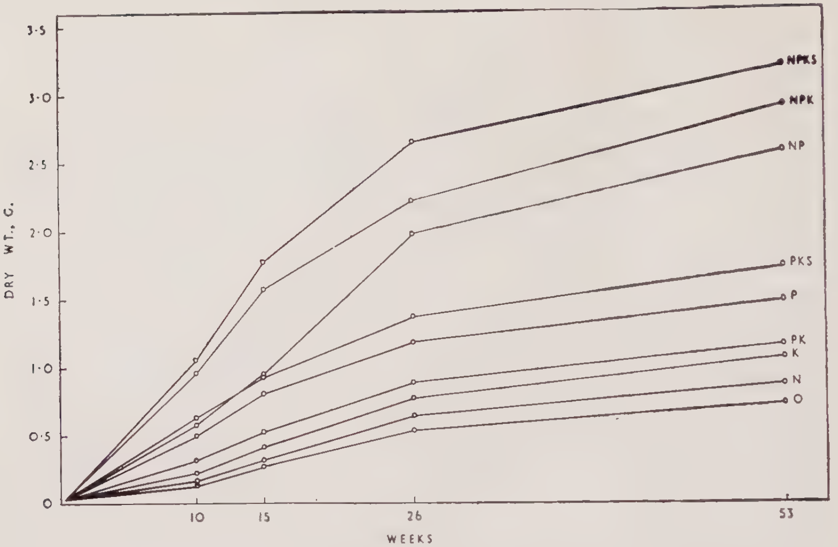


Fig. 1.—Cumulative dry weights of leaves of plants of *Festuca novae-zelandiae* receiving various fertilisers. (Means of 8 plants.)

position in a glasshouse. Leaves were harvested by cutting $\frac{3}{4}$ in. above the soil surface and dried to a constant weight at 105°c. At each harvest the mean dry weight was added to those from preceding harvests and the cumulative weights from each of the four harvests are presented in Fig. 1.

RESULTS AND DISCUSSION

In general, treatments that lacked phosphorus were hardly more effective than untreated controls. The response to phosphorus by itself was immediate but was not continued beyond the second harvest. However, after one year the crop yield was double that of untreated controls.

The effect of potassium alone was slight and had disappeared after the third harvest.

Nitrogen by itself, or in combination with potassium, or potassium plus sulphur, had little effect, but over the experimental period of one year the combination of nitrogen and phosphorus produced a four- to five-fold increase in crop yields over the untreated controls. It would, therefore, appear that in this soil although the level of nitrogen available to plants is low, as indicated by the nitrification experiments of Ross (1958), the response to added nitrogen fertiliser depends on the simultaneous addition of phosphorus.

The addition of potassium to nitrogen plus phosphorus treatments gave a further response.

The addition of sulphur to the potassium plus phosphorus treatment caused an increase in growth at the first harvest which was not substantially increased in later harvests. The addition of sulphur to the nitrogen plus phosphorus plus potassium treatment, however, caused an increase in growth which was very marked at the third harvest and showed a decline at the fourth. Walker *et al.* (1955) showed that clover, in field trials in tussock grasslands, responded to the addition of sulphur.

REFERENCES

ROSS, D. J. 1958: Biological Studies of Some Tussock-grassland Soils. VI. Nitrifying Activities. *N.Z. J. agric. Res. 1:* 968-73.

THORNTON, R. H. 1958: Biological Studies of Some Tussock-grassland Soils. I. Introduction, Soils and Vegetation. *Ibid. 1:* 913-21.

WALKER, T. W.; ADAMS, A. F. R.; ORCHISTON, H. D. 1955: The Effects and Interactions of Sulphur, Phosphorus, and Molybdenum on the Growth and Composition of Clovers. *N.Z. J. Sci. Tech. A36:* 470-82.

CORRIGENDUM

Volume 1, page 646, line 4, *for* aecidiospores *read* uredospores.

INDEX

VOLUME 1

Page

Anderson, J. P. (see Andrews, E. D. *et al*) 125
Andrews, E. D.; Stephenson, B. J.; Anderson, J. P.; Faithful, W. C.: The effect of length of pastures on cobalt-deficiency disease in lambs 125
Aphid counter, The development of an electronic, 903
Apple mosaic, Transmission by natural root grafting 80
Apple trees, Effect of magnesium topdressings on young, 796
Apples, Superficial scald: some methods of control, reported overseas, applied to New Zealand Granny Smith, 231
Askew, H. O.: Molybdenum in relation to the occurrence of xanthin calculi in sheep 447
Askew, H. O.: Chittenden, E.: The effect of magnesium topdressings on the growth of young apple trees 796
Askew, H. O.; Monk, R. J.; Watson, Joyce: Molybdenum deficiency of the hop 553
Askew, H. O.; Monk, R. J.; Watson, Joyce: Physical and chemical changes in the developing hop cone 757
Askew, H. O.; Chittenden, E.; Watson, Joyce: Waters, A. J.: Influence of applications of sodium molybdate and ground limestone on a newly sown pasture 874
Atkinson, J. D. (see Hunter, J. A. *et al.*) 80

Bailey, R. W.: Bloat in cattle. X. The carbohydrases of the cattle rumen ciliate *Epidinium ecaudatum* Crawley isolated from cows fed on red clover (*Trifolium pratense* L.) 825
Barton, R. A. (see Kirton, A. H.) 633
Barton, R. A.; Kirton, A. H.: The leg and the loin as indices of the composition of New Zealand lamb and mutton carcasses 783
Bathurst, N. O.; Mitchell, K. J.: The effect of light and temperature on the chemical composition of pasture plants 540
Big-vein disease of lettuce, The relationship of *Olpidium brassicae* to, 301
Biological studies of some tussock-grassland soils:
  I. Introduction, soils and vegetation 913
  II. Fungi 922
  III. Yeasts 939
  IV. Bacteria 943
  V. Non-symbiotic nitrogen-fixing bacteria 958
  VI. Nitrifying activities 968
  VII. Protozoa 974
  VIII. Streptomycetes 985
  IX. Algae 991
  X. Earthworms 993
  XI. Response of a tussock plant, *Festuca novae-zelandiae*, to fertilisers 1003
Bloat in cattle:
  VII. The measurement of foaming properties of surface-active compounds 140
  VIII. Prevention by pasture spraying with anti-foaming agents 349
  IX. Some observations on the culture of the cattle rumen ciliate *Epidinium ecaudatum* Crawley occurring in quantity in cows fed on red clover (*Trifolium pratense* L.) 809
  X. The carbohydrases of the cattle rumen ciliate *Epidinium ecaudatum* Crawley isolated from cows fed on red clover (*Trifolium pratense* L.) 825
Boyce, W. R.: Resistance to yellow-leaf disease in *Phormium tenax* Forst., and observations on delayed expression of symptoms 31
Brevicoryne brassicae (L). Effect of Metasystox on, 37

Page

Brougham, R. W.: Leaf development in swards of white clover (*Trifolium repens* L.) 707

Calder, D. M. (see Mitchell, K. J.) 61
Carcasses, Specific gravity as an index of the fat content of mutton . . . and various joints 633
Carcasses, The leg and the loin as indices of the compostion of New Zealand lamb and mutton, 783
Carotene, Effect of tocopherol on the utilisation of intravenously administered, 273
Carotene, Effects of paraffins and other substances on the utilisation of high dosage levels of, 267
Case-bearers, A review of work in New Zealand on the clover . . . *Coleophora spissicornis* and *C. alcyonipennella?* Kollar 239
Cattle, Seasonal growth relationships in Aberdeen Angus × Jersey crossbred, 341
Chamberlain, E. E. (see Hunter, J. A. *et al.*) 80
Characteristic filamentous bacterial rumen organism indicative of a sustained low ruminal ammonia-nitrogen level 17
Chittenden, E. (see Askew, H. O.) 796, 874
Chlorpromazine in the measurement of radioactive iodine in the thyroid gland of the rat 455
Clarke, E. A.; Filmer, Daisy B.: Studies in hogget rearing:
  I. General characteristics of hogget ill-thrift 249
  II. The role of parasites in hogget ill-thrift 382
"Cloud" or vascular browning in tomatoes. IV. Polyphenol oxidase activity in cloud-susceptible fruit 896
Clover case-bearer caterpillars, Damage by, 525
Cobalt-deficiency disease in lambs, Effect of length of pastures on, 125
Cocksfoot (*Dactylis glomerata* L.), Fungal leaf-spot diseases of 182, 800
Coles, S. T. J. (see Edmond, D. B.) 665
Collar rot and root rot of Cox's Orange Pippin and other apple varieties in Nelson and Marlborough 462
Comparison of phosphatic fertilisers 418
Concentration of radioactive iodine by the thyroid glands of normal sheep under various conditions 224
Control of lice (*Damalinia ovis*) and keds (*Melophagus ovinus*)—a comparison of the efficiency of common insecticides 217
Control of *Pieris rapae* by granulosis viruses 778
Coop, I. E.; Clark, V. R.: The effect of thyroxine on wool growth and lamb production 365
Copper content of hair, hoof, and wool 841
Cullen, N. A.: A comparison of phosphatic fertilisers 418
Cumber, R. A.: The insect complex of sown pastures in the North Island. I. The general picture revealed by summer sweep-sampling 719
Cunningham, I. J.: Non-toxicity to animals of ryegrass endophyte and other endophytic fungi of New Zealand grasses 489
Cunningham, I. J.: Housing a guinea-pig colony 750
Cunningham, I. J.; Hogan, K. G.: The influence of diet on the copper and molybdenum contents of hair, hoof, and wool 841

Dactylis glomerata L., Fungal leaf-spot diseases of, 182, 800
Damage by clover case-bearer caterpillars 525
Damage in ryegrasses by *Hyperodes griseus* Hust. 790
DDT, Effect on honey bees of DDT applied from the air as a spray to lucerne 627
Development of an electronic aphid counter 903
Development of streptomycin-resistant variants of *Pseudomonas syringae* van Hall in culture and in peach seedlings 44
Driver, C. M.: Races of potato blight, *Phytophthora infestans* (Mont.) de Bary, in New Zealand 527

Page

Dromgoole, W. V. (see Lowe, A. D.) 903
Dunkin, A. C.: The use of condensed whey as a food for fattening pigs 531
Dye, M. H.: Development of streptomycin-resistant variants of *Pseudomonas syringae* van Hall in culture and in peach seedlings 44
Edmond, D. B.: Some effects of MCPB on establishing pastures 281
Edmond, D. B.: Some effects of soil physical condition on ryegrass growth 652
Edmond, D. B.: The influence of treading on pastures: a preliminary study 319
Edmond, D. B.; Coles, S. T. J.: Some long-term effects of fertilisers on a mown turf of browntop and Chewings fescue 665
Eelworm (*Anguina agrostis* (Steinb.) Filipjer) in *Agrostis tenuis* Sibth. in New Zealand 265
Effect of differences in light intensity and temperature on the anatomy and development of leaves of *Lolium perenne* and *Paspalum dilatatum* 1
Effect of different phosphate fertilisers on soil pH and the consequent effect on phosphate retention 675
Effect of length of pastures on cobalt-deficiency disease in lambs 125
Effect of light and temperature on the chemical composition of pasture plants 540
Effect of magnesium topdressings on the growth of young apple trees 796
Effect of Metasystox on cabbage aphid (*Brevicoryne brassicae* (L.)) 37
Effect of several fungicides on stem rot of tobacco 866
Effect of stilbestrol implants upon the growth and fattening of Romney wether lambs 69
Effect of thyroxine on wool growth and lamb production 365
Effect of tocopherol on the utilisation of intravenously administered carotene 273
Effect on honey bees of DDT applied from the air as a spray to lucerne. Notes on lucerne pollination 627
Effect on honey bees of some defoliants 620
Effect on honey bees of toxaphene and strobane applied to white-clover pasture 694
Effectiveness of honey bees and bumble bees in the pollination of Montgomery red clover 607
Effects of flowering on the root system and summer survival of ryegrass 329
Effects of ingestion of paraffins by ruminants. IV. Effects of paraffins and other substances on the utilisation of high dosage levels of carotene 267
Elliott, I. L.; Lynch, P. B.: Techniques of measuring pasture production in fertiliser trials 498
Epidinium ecaudatum in cows on red clover 809, 825
Examination of the surface-active agent PR 51 as an accelerator of drainage from pasture topsoils 834

Facial-eczema grass, Substances isolated from concentrates of, 859
Facial-eczema poison, Concentration of, 433
Faithful, W. C. (see Andrews, E. D. *et al.*) 125
Fejer, S. O.: Genetic and environmental components of the productivity of perennial ryegrass (*Lolium perenne* L.) 86
Fertilisers, Some long-term effects on a mown turf of browntop and and Chewings fescue 665
Filmer, Daisy B. (see Clark, E. A.) 249, 382
Flint, A. E. Biological studies of some tussock-grassland soils. IX. Algae. Preliminary observations 991
Forster, I. W. (see Palmer-Jones, T.) 620, 627, 694
Forster, I. W.; Hadfield, V. W.: Effectiveness of honey bees and bumble bees in the pollination of Montgomery red clover 607
Fry, P. R.: The relationship of *Olpidium brassicae* (Wor.) Dang. to the big-vein disease of lettuce 301

Page

Fungal leaf-spot diseases of cocksfoot (*Dactylis glomerata* L.) in the Manawatu:
    I. Leaf streak caused by *Scolecotrichum graminis* Fckl. 182
    II. Purple leaf spot caused by *Stagonospora maculata* (Grove) Sprague, and leaf fleck caused by *Mastigosporium rubricosum* (Dearn. et Barth.) Sprague 800

Genetic and environmental components of the productivity of perennial ryegrass (*Lolium perenne* L.) 86
Genetic variation in New Zealand Romney Marsh sheep 104
Glenday, A. C. (see Mitchell, K. J.) 305
Goitrogenic effect of thousand-headed kale on adult sheep and rabbits 477
Gradwell, M. W.: An examination of the surface-active agent PR 51 as an accelerator of drainage from pasture topsoils 834
Granulosis viruses, Control of *Pieris rapae* by, 778
Grasses, Potassium content of legumes and, 148
Greenall, A. F.: Studies of grazing crop forages by sheep:
    I. The effect of grazing on composition, digestibility, and nutritive value of rape 569
    II. Wastage of rape by grazing wethers 683
Guinea-pig colony, Housing a, 750

Hadfield, W. V. (see Forster, I. W.) 607
Healy, A. J.: Eelworm (*Anguina agrostis* (Steinb.) Filipjer) in *Agrostis tenuis* Sibth. in New Zealand 265
Hogan, K. G. (see Cunningham, I. J.) 841
Hogget ill-thrift 249, 382
Honey bees, Effect of DDT spray on, 627
Honey bees, Effect of some defoliants on, 620
Honey bees, Effect of toxaphene and strobane on, 694
Honey bees, Laboratory methods for measuring the toxicity of pesticides to, 290
Hop, Molybdenum deficiency of the, 553
Hop, Physical and chemical changes in the developing cone 757
Housing a guinea-pig colony 750
Hunter, J. A.; Chamberlain, E. E.; Atkinson, J. D.: Note on transmission of apple mosaic by natural root grafting 80
Hyperodes griseus Hust., Damage in ryegrasses 790

Ill-thrift, General characteristics of hogget, 249
Ill-thrift, The role of parasites in hogget, 382
Incidence and parasitism of insect pests of cruciferous crops in Hawke's Bay, Manawatu, Rangitikei, and Taranaki, 1956–57 847
Influence of applications of sodium molybdate and ground limestone on a newly sown pasture 874
Influence of diet on the copper and molybdenum contents of hair, hoof, and wool 841
Influence of treading on pastures: a preliminary study 319
Insect complex of sown pastures in the North Island. I. The general picture revealed by summer sweep-sampling 719

Jacques, W. A. (see Robinson, G. S.) 199
Jafar, Hassan: Investigation on the control of peach rust (*Tranzschelia pruni-spinosae* Pers.) in New Zealand 660
Jafar, Hassan: Studies on the biology of peach rust (*Tranzschelia pruni-spinosae* Pers.) in New Zealand 642
Jamieson, N. D.; Loftus, T. M.: A characteristic filamentous bacterial rumen organism indicative of a substained low ruminal ammonia-nitrogen level 17

Karlovsky, J: Pasture responses to phosphatic fertilisers 51
Kelsey, J. M.: Control of *Pieris rapae* by granulosis viruses 778

Page

Kelsey, J. M.: Damage by clover case-bearer caterpillars 525
Kelsey, J. M.: Damage in reygrasses by *Hyperodes griseus* Hust. 790
Kidson Elsa B.: “Cloud or vascular browning in tomatoes. IV. Polyphenol oxidase activity in cloud-susceptible fruit 896
Kirton, A. H. (see Barton, R. A.) 783
Kirton, A. H. (see Munford, R. E.) 83
Kirton, A. H.; Barton, R. A.: Specific gravity as an index of the fat content of mutton carcasses and various joints 633

Laboratory methods for measuring the toxicity of pesticides to honey bees 290
Lambourne, L. J.: The effect of stilbestrol implants upon the growth and fattening of Romney wether lambs 69
Lambs, The effect of length of pastures on cobalt-deficiency disease in 125
Latch, G. C. M. (see Wenham, H. T.) 800
Latch, G. C. M.; Wenham, H. T.: Fungal leaf-spot diseases of cocksfoot (*Dactylis glomerata* L.) in the Manawatu. I. Leaf streak caused by *Scolecotrichnm graminis* Fckl. 182
Leaf development in swards of white clover (*Trifolium repens* L.) 707
Lee, K. E.: Biological studies of some tussock-grassland soils. X. Earthworms 998
Leg and loin as indices of the composition of New Zealand lamb and mutton carcasses 783
Legumes and grasses, Potassium content of, 148
Light regime within pastures 61
Line, L. J. S. (see Palmer-Jones, T.) 694
Loftus, T. M. (see Jamieson, N. D.) 17
Lolium perenne L., Genetic and environmental components of the productivity of, ... 86
Lolium perenne and *Paspalum dilatatum*, Effects of differences in light intensity and temperature on the anatomy and development of leaves of, ... 1
Lowe, A. D.: Effect of Metasystox on cabbage aphid (*Brevicoryne brassicae* (L.)) 37
Lowe, A. D.: Dromgoole, W. V.: The development of an electronic aphid counter 903
Lynch, P. B. (see Elliott, I. L.) 498

MacDonald, M. A.: Seasonal growth relationships in Aberdeen Angus × Jersey crossbred cattle 341
McGillivray, W. A.; Worker, N. A.: Effects of ingestion of paraffins by ruminants. IV. Effects of paraffins and other substances on the utilisation of high dosage levels of carotene 267
McGillivray, W. A. Worker, N. A.: The effect of tocopherol on the utilisation of intravenously administered carotene 273
McLeod, A. G.; Thomson, R.: The effect of several fungicides on stem rot of tobacco 866
McNaught, K. J.: Potassium deficiency in pastures. I. Potassium content of legumes and grasses 148
Magnesium topdressings, Effect on young apple trees 796
Mangan, J. L.: Bloat in cattle. VII. The measurement of foaming properties of surface-active compounds 140
MCPB, Some effects on establishing pastures .. 281
Menna, M. E. di: Biological studies of some tussock-grassland soils. III. Yeasts 939
Metasystox, Effect on cabbage aphid (*Brevicoryne brassicae*) ... 37
Mitchell, K. J. (see Bathurst, N. O.) 540
Mitchell, K. J.; Calder, D. M.: The light regime within pastures 61
Mitchell, K. J.; Glenday, A. C.: The tiller population of pastures 305
Mitchell, K. J.; Soper, Kathleen: Effects of differences in light intensity and temperature on the anatomy and development of leaves of *Lolium perenne* and *Paspalum dilatatum* .. 1

Page

Molybdate, Influence on newly sown pasture 874
Molybdenum content of hair, hoof, and wool 841
Molybdenum deficiency of the hop 553
Molybdenum in relation to the occurrence of xanthin calculi in sheep 447
Monk, R. J. (see Askew, H. O.) 553, 757
Morrison, T. M.: Biological studies of some tussock-grassland soils. XI. The response of a tussock plant, *Festuca novae-zelandiae*, to fertilisers 1003
Munford, R. E.; Kirton, A. H.: Thyroid follicular cell height 83

Non-toxicity to animals of ryegrass endophyte and other endophytic fungi of New Zealand grasses 489
Note on transmission of apple mosaic by natural root grafting 80

O'Grady, B. (see Thomas, P. L.) 522
Olpidium brassicae, Relationship to big-vein disease of lettuce 301
Oxford, A. E.: Bloat in cattle. IX. Some observations on the culture of the cattle rumen ciliate *Epidinium ecaudatum* Crawley occurring in quantity in cows fed on red clover (*Trifolium pratense* L.) 809

Padfield, C. S.: Superficial scald: some methods of control, reported overseas, applied to New Zealand-grown Granny Smith apples 231
Palmer-Jones, T.: Laboratory methods for measuring the toxicity of pesticides to honey bees 290
Palmer-Jones, T; Forster, I. W.: Effect on honey bees of DDT applied from the air as a spray to lucerne. Notes on lucerne pollination 627
Palmer-Jones, T.; Forster, I. W.: Effect on honey bees of some defoliants 620
Palmer-Jones, T.; Forster, I. W.; Line, L. J. S.: Effect on honey bees of toxaphene and strobane applied to white-clover pasture 694
Paraffins, Effects of ingestion by ruminants 267
Paspalum dilatatum, Effects of differences in light intensity and temperature on the anatomy and development of leaves of *Lolium perenne* and, ... 1
Pasture responses to phosphatic fertilisers 51
Peach rust, Studies on the biology of, ... 642
Peach rust, Investigation on the control of, ... 660
Phormium tenax Forst., Resistance to yellow-leaf disease in . . . , and observations on delayed expression of symptoms 31
Phosphate retention, The effect of different phosphate fertilisers on soil pH and the consequent effect on, 675
Phosphatic fertilisers, A comparison of, ... 418
Phosphatic fertilisers, Pasture responses to, ... 51
Photosensitivity diseases in New Zealand:
  XII. Concentration of the facial-eczema poison 433
  XIII. Substances isolated from concentrates of facial-eczema grass 859
Physical and chemical changes in the developing hop cone 757
Phytophthora infestans (Mont.) de Bary in New Zealand, Races of, 527
Pigs, The use of condensed whey as a food for fattening, ... 531
Pollination of Montgomery red clover, Effectiveness of honey bees and bumble bees in, ... 607
Potassium deficiency in pastures. I. Potassium content of legumes and grasses 148
PR 51 as an accelerator of drainage 834
Pseudomonas syringae van Hall, Development of streptomycin-resistant variants in culture and in peach seedlings 44

Quick method for applying insecticide dust to experimental small animals 522

Races of potato blight, *Phytophthora infestans* (Mont.) de Bary, in New Zealand 527
Rae, A. L.: Genetic variation in New Zealand Romney Marsh sheep 104

Page

Rape, The effect of grazing on composition, digestibility, and nutritive value of, ... 569
Rape, Wastage by grazing wethers 683
Reid, C. S. W.: Bloat in cattle. VIII. Prevention by pasture spraying with anti-foaming agents 349
Relationship of *Olpidium brassicae* (Wor.) Dang. to the big-vein disease of lettuce 301
Resistance to yellow-leaf disease in *Phormium tenax* Forst., and observations on delayed expression of symptoms 31
Review of work in New Zealand on the clover case-bearers *Coleophora spissicornis* Haw. and *C. alcyonipennella* ? Kollar (Coleophoridae, Lep.) 239
Robinson, G. S.; Jacques, W. A.: Root development in some common New Zealand grasses. X. Effect of pure sowings of some grasses and clovers on the structure of a Tokomaru silt loam 199
Root development in some common New Zealand pasture plants. X. Effect of pure sowings of some grasses and clovers on the structure of a Tokomaru silt loam 199
Ross, D. J.: Biological studies of some tussock-grassland soils:
  V. Non-symbiotic nitrogen-fixing bacteria 958
  VI. Nitrifying activities 968
Rumen organism, A characteristic filamentous bacterial . . . indicative of a sustained low ruminal ammonia-nitrogen level 17
Ryegrass, Effects of flowering on the root system and summer survival of, ... 329
Ryegrass endophyte, Non-toxicity to animals 489

Saunders, W. M. H.: The effect of different phosphate fertilisers on soil pH and the consequent effect on phosphate retention 675
Seasonal growth relationships in Aberdeen Angus × Jersey crossbred cattle 341
Septoria drummondii Ell. et Ev., a common seed-borne pathogen in European lines of *Phlox drummondii* Hook. seed imported to New Zealand 486
Sheep, Genetic variation in New Zealand Romney Marsh, ... 104
Sheep, Studies on the thickness and chemical composition of the skin of, ... 582
Sheep, Surface applicators for the control of lice and keds on 189
Sheep, The concentration of radioactive iodine by the thyroid glands of normal, ... 224
Soils, Tussock-grassland (see Biological studies of,) 913
Some effects of MCPB on establishing pastures 281
Some effects of soil physical condition on ryegrass growth 652
Some long-term effects of fertilisers on a mown turf of browntop and Chewings fescue 665
Soper, Kathleen: Effects of flowering on the root system and summer survival of ryegrass 329
Soper, Kathleen (see Mitchell, K. J.) 1
Stephenson, B. J. (see Andrews, E. D. *et al.*) ... 125
Stilbestrol, The effect of . . . implants upon the growth and fattening of Romney wether lambs 69
Stout, J. D.: Biological studies of some tussock-grassland soils:
  IV. Bacteria 943
  VII. Protozoa 974
Specific gravity as an index of the fat content of mutton carcasses and various joints 633
Strobane, Effect on honey bees, ... 694
Stuart, A. M.: A review of work in New Zealand on the clover case-bearers *Coleophora spissicornis* Haw. and *C. alcyonipennella?* Kollar (Coleophoridae, Lep.) 239
Studies in hogget rearing:
  I. General characteristics of hogget ill-thrift 249
  II. The role of parasites in hogget ill-thrift 382

Page

Studies of grazing crop forages by sheep:
    I. The effect of grazing on composition, digestibility, and nutritive value of rape 569
    II. Wastage of rape by grazing wethers 683
Studies on the biology of peach rust (*Tranzschelia pruni-spinosae* Pers.) in New Zealand 642
Studies on the thickness and chemical composition of the skin of sheep 582
Superficial scald: some methods of control, reported overseas, applied to New Zealand-grown Granny Smith apples 231
Surface applicators for the control of lice and keds on sheep 189
Survey of collar rot and root rot of Cox's Orange Pippin and other apple varieties in Nelson and Marlborough 462

Techniques of measuring pasture production in fertiliser trials 498
Thomas, P. L.: Surface applicators for the control of lice and keds on sheep 189
Thomas, P. L.: The control of lice (*Damalinia ovis*) and keds (*Melophagus ovinus*)—a comparison of the efficiency of common insecticides 217
Thomas, P. L.; O'Grady, B.: A quick method for supplying insecticide dust to experimental small animals 522
Thomson, R. (see McLeod, A. G.) 866
Thornton, R. H.: Biological studies of some tussock-grassland soils:
    I. Introduction, soils and vegetation 913
    II. Fungi 922
Thousand-headed kale, Goitrogenic effect on adult sheep and rabbits 477
Thyroid follicular cell height 83
Thyroid glands of normal sheep, Concentration of radioactive iodine by the, ... 224
Thyroxine, Effect on wool growth and lamb production 365
Tiller population of pastures 305
Tobacco, The effect of several fungicides on stem rot of, ... 866
Tocopherol, Effect on the utilisation of intravenously administered carotene 273
Todd, D. H.: Incidence and parasitism of insect pests of cruciferous crops in Hawke's Bay, Manawatu, Rangitikei, and Taranaki, 1956–57 847
Tomatoes, "Cloud" or vascular browing in, ... 896
Toxaphene, Effect on honey bees, ... 694
Tranzschelia pruni-spinosae in New Zealand, Studies on the biology of, ... 642
Tranzschelia pruni-spinosae, Investigation on the control of, ... 660
Trials, Techniques of measuring pasture production in fertiliser, 498
Trifolium repens L., Leaf development in swards of, ... 707
Tussock-grassland soils (see Biological studies of,) 913

Use of chlorpromazine in the measurment of radio-active iodine in the thyroid gland of the rat 455
Use of condensed whey as a food for fattening pigs 531

Vernon, T. R.: Biological studies of some tussock-grassland soils. VII. Streptomycetes 985

Waters, A. J. (see Askew, H. O.) 874
Watson, Joyce (see Askew, H. O.) 553, 757, 874
Wenham, H. T.: *Septoria drummondii* Ell. et Ev., a common seed-borne pathogen in European lines of *Phlox drummondii* Hook. seed imported to New Zealand 486
Wenham, H. T. (see Latch, G. C. M.) 182
Wenham, H. T.; Latch, G. C. M.: Fungal leaf-spot diseases of cocksfoot (*Dactylis glomerata* L.) in the Manawatu. II. Purple leaf-spot caused by *Stagonospora maculata* (Grove) Sprague, and leaf fleck caused by *Mastigosporium rubricosum* (Dearn. et Barth.) Sprague 800

Page

Whey as a food for fattening pigs, The use of condensed, 531
White, E. P.: Photosensitivity diseases in New Zealand:
 XII. Concentration of the facial-eczema poison 433
 XIII. Substances isolated from concentrates of facial-eczema grass 859
Wodzicka, Manika: Studies on the thickness and chemical composition of the skin of sheep 582
Woodhead, C. E.: A survey of collar rot and root rot of Cox's Orange Pippin and other apple varieties in Nelson and Marlborough 462
Worker, N. A. (see McGillivray, W. A.) 267, 273
Wright, Evan: The concentration of radioactive iodine by the thyroid glands of normal sheep under various conditions ... 224
Wright, Evan: The use of chlorpromazine in the measurement of radioactive iodine in the thyroid gland of the rat 455
Wright, Evan: Sinclair, D. P.: The goitrogenic effect of thousand-headed kale on adult sheep and rabbits 477

Xanthin calculi in sheep, Molybdenum in relation to occurrence of. 447

NEW ZEALAND
JOURNAL OF SCIENCE

Vol. 1, No. 4, December 1958

CONTENTS

Racial Differences in Size and Growth in the New Zealand Snapper. *Alan R. Longhurst*

A Reconnaisance Survey of Pollution in the Manawatu River. *J. K. Johanneson*

The Occurrence of an Argasid Tick in New Zealand. *L. J. Dumbleton*

Alate Aphids Trapped in Auckland, New Zealand, using Moericke Colour Traps. *K. P. Lamb*

Note on the Efficiency of Aphid Trapping. *H. R. Thompson*

Biological Evaluation of Organic Pollution of New Zealand Streams. *Allan Hirsch*

Contributions to a Chromosome Atlas of the New Zealand Flora. I. *J. B. Hair*

Foods of Australian Opossums (*Trichosurus vulpecula* Kerr) in New Zealand Indigenous Forest in the Orongorongo Valley, Wellington. *R. Mason*

Index to Volume 1.